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## NEW BIOLOGICAL DATA ON THE EAGLE RAY, *MYLIOBATIS AQUILA* (CHONDRICHTHYES: MYLIOBATIDAE), OFF THE LANGUEDOCIAN COAST (SOUTHERN FRANCE, NORTHERN MEDITERRANEAN)

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### ABSTRACT

*Investigations conducted off the Languedocian coast allowed the authors of the paper to capture 73 specimens of the eagle ray *Myliobatis aquila* (Linnaeus, 1758) and to obtain information on hepatosomatic index (HSI) and gonosomatic index (GSI) in males and females. Relationships between disc width (DW) and HSI, and DW and GSI did not show significant differences between males and females. However, the highest value of HSI and GSI were recorded in both sub-adult and adult specimens, especially in adult females showing the role of the liver in the gonadal production as well as in buoyancy.*

**Key words:** Chondrichthyes, Myliobatidae, *Myliobatis aquila*, liver, gonads, Languedocian coast, Mediterranean

## NUOVI DATI BIOLOGICI PER L'AQUILA DI MARE, *MYLIOBATIS AQUILA* (CHONDRICHTHYES: MYLIOBATIDAE), AL LARGO DELLA COSTA DI LANGUEDOC (FRANCIA MERIDIONALE, MEDITERRANEO SETTENTRIONALE)

### SINTESI

*Gli autori riportano la cattura di 37 esemplari di Aquila di mare, *Myliobatis aquila* (Linnaeus, 1758), durante una ricerca al largo della costa di Languedoc. Sono state così ottenute informazioni sull'indice epatosomatico (HSI) e sull'indice gonosomatico (GSI) sia nei maschi che nelle femmine della specie. Le relazioni fra la larghezza del disco (DW) e l'HSI, e fra DW e GSI, non hanno evidenziato importanti differenze fra i sessi. I valori più elevati di HSI e GSI, comunque, sono stati registrati in individui quasi adulti ed adulti, principalmente femmine, il che evidenzia il ruolo del fegato nella produzione gonadica e sulla galleggiabilità.*

**Parole chiave:** Chondrichthyes, Myliobatidae, *Myliobatis aquila*, fegato, gonadi, Languedoc, Mediterraneo

## INTRODUCTION

According to McEachran & Capapé (1984), the common eagle ray *Myliobatis aquila* (Linnaeus, 1758) is a typical Atlanto-Mediterranean species. It has been reported from off Scandinavia (Muus & Dahlstrøm, 1964-1966) and British Isles (Wheeler, 1969) to Portugal (Albuquerque, 1954-1956), south of the Strait of Gibraltar, from off Morocco (Collignon & Aloncle, 1972) and Mauritania (Maurin & Bonnet, 1970) to Senegal (Cadenat, 1951). It is known throughout the Mediterranean and has recently been reported from the eastern Levantine Basin by Golani (2005).

From off the Languedocian coast, *M. aquila* was formerly reported by various authors, from Doumet (1860) to Quignard *et al.* (1962). In this area, the species was not considered as abundantly landed; due to their low economical interest, eagle rays were generally discarded at sea by fishermen soon after capture. However, investigations carried out in the area allow Capapé *et al.* (2007) to collect several specimens and to present some traits of the reproductive biology of the species, such as size at sexual maturity, reproductive cycle and fecundity, which were compared with similar data recorded from specimens off the Tunisian coast (Capapé & Quignard, 1974).

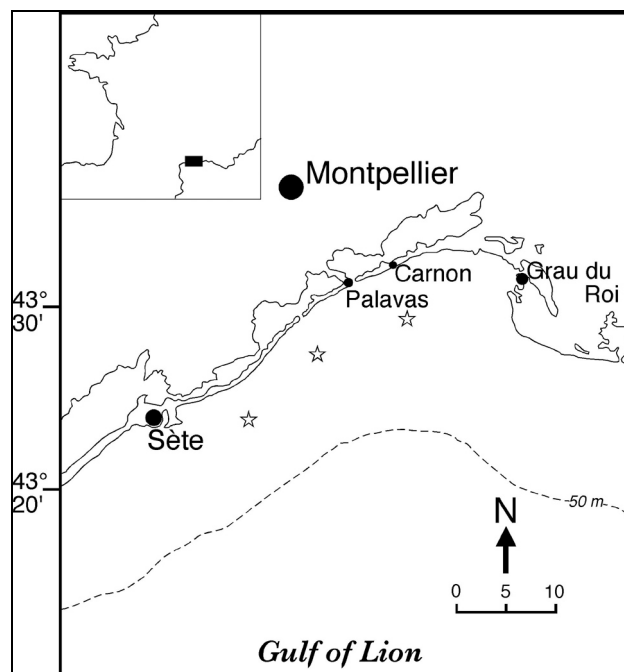
In the present paper, we provide additional observations on *M. aquila* by analyzing variations of gonadosomatic and hepatosomatic indexes in both sexes, in order to detect seasonal variations in the gonadal production. Our results are compared and contrasted with those carried out in the viviparous angular rough shark *Oxynotus centrina* (Linnaeus, 1758) and the oviparous smallspotted catshark *Scyliorhinus canicula* (Linnaeus, 1758) from the same area (Capapé *et al.*, 1999, 2008).

## MATERIAL AND METHODS

Between 1990 and 2004, specimens were captured by demersal gill-nets at depths from 30 to 40 m, between Sète and Le Grau-du-Roi (Fig. 1). Disc width (DW) of the specimens was measured to the nearest millimetre following Clark (1926) and mass (TM) to the nearest gram, liver, gonads and the masses of oviducal glands to the nearest decigram.

Three stages of male maturity were considered relative to the degree of calcification of claspers and the morphology of the genital duct, following Capapé *et al.* (2007). They were juvenile, sub-adult and adult. Similar stages were also considered in females from the condition of ovaries, the morphology of the reproductive tract following Callard *et al.* (2005), Henderson *et al.* (2006) and Capapé *et al.* (2007). Hepatosomatic index (HSI) and gonadosomatic index (GSI) were calculated in both males and females as  $HSI = (LM/TM) * 100$ ,  $GSI = (GM/TM) * 100$ . Variations in GSI and HSI were consid-

ered in all categories of specimens in both sexes. Tests for significance ( $p < 0.05$ ) were performed by using ANOVA, with special regard to variations in HSI and GSI and related to size.



**Fig. 1: Map of France pointing with the coast of Languedoc and captures sites (stars) of the eagle ray *Myliobatis aquila* in the area (redrawn from Capapé *et al.*, 2000).**

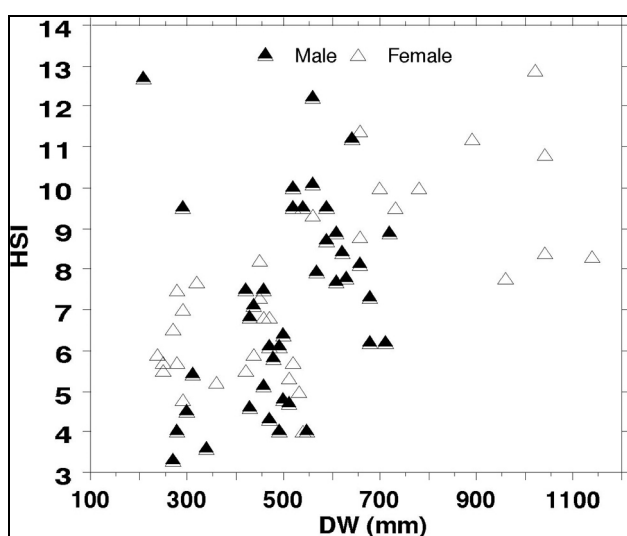
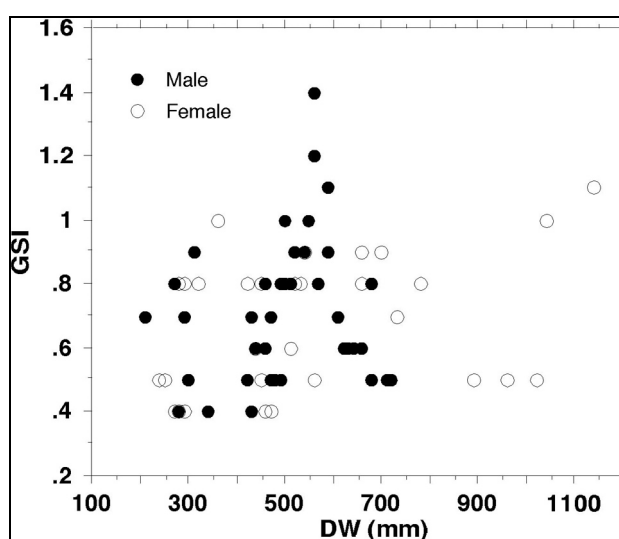
**Sl. 1: Zemljevid Francije z obrežnimi vodami pokrajine Languedoc in lokalitetami (zvezdice), kjer so bili ujeti navadni morski golobi *Myliobatis aquila* (narisano po Capapé *et al.*, 2000).**

## RESULTS

In all, 73 common eagle rays were observed, 41 were males and 32 females. The monthly collection of the observed specimens is summarized in Table 1. Juvenile males comprised 16 specimens, with their disc width ranging between 210 and 490 mm and weight between 166 and 1495 g. Juveniles were generally caught between August and October, one specimen was caught in March, one in May. Eight sub-adults were collected, seven between August and October and a single specimen in May. The smallest sub-adult observed was 460 mm DW and weighing 1600 g, the largest 520 mm DW and weighing 1780 g. The smallest adult male was 500 mm DW and weighing 2,000 g, the largest was 720 mm DW and weighing 5,250 g, while the heaviest specimen weighed 5,460 g and was 710 mm DW. All males above 540 mm DW were adult.

**Tab. 1: Monthly collection of the observed *M. aquila* captured off the coast of Languedoc.****Tab. 1: Mesečna zbirka navadnih morskih golobov *M. aquila*, ujetih v obrežnih vodah pokrajine Languedoc.**

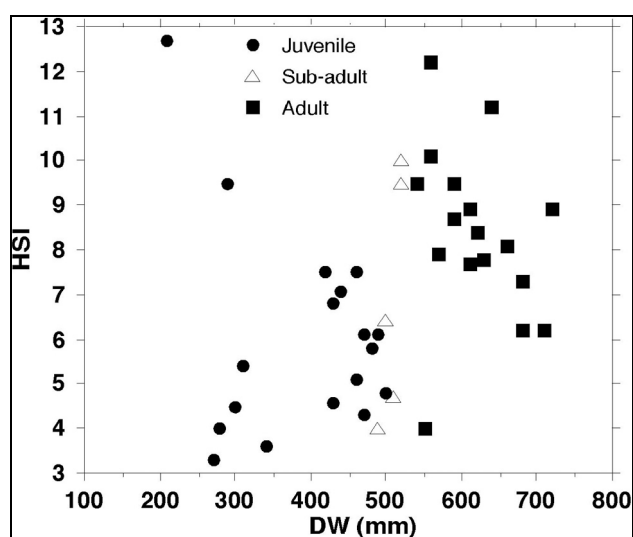
Sex	Category	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
Males	Juveniles	-	-	1	-	-	-	-	5	6	4	-	-	16
	Sub-adults	-	-	-	-	1	-	-	2	4	1	-	-	8
	Adults	-	-	2	-	4	-	-	3	2	6	-	-	17
	Total	-	-	3	-	5	-	-	10	12	11	-	-	41
Females	Juveniles	-	-	1	-	-	-	-	2	11	2	-	-	16
	Sub-adults	-	-	2	-	-	-	-	2	1	3	-	-	8
	Adults	1	-	1	-	-	-	-	2	3	1	-	-	8
	Total	1	-	4	-	-	-	-	6	15	6	-	-	32
<b>Grand total</b>		<b>1</b>	<b>-</b>	<b>7</b>	<b>-</b>	<b>5</b>	<b>-</b>	<b>-</b>	<b>16</b>	<b>27</b>	<b>17</b>	<b>-</b>	<b>-</b>	<b>73</b>

**Fig. 2: Relationship between hepatosomatic index (HSI) and disc width (DW) for both male and female *M. aquila* from the Languedocian coast.****Sl. 2: Razmerja med hepatosomatskim indeksom (HSI) in širino diska (DW) pri samcih in samicah vrste *M. aquila*, ujetih v obrežnih vodah pokrajine Languedoc.****Fig. 3: Relationship between gonosomatic index (GSI) and disc width (DW) for both male and female *M. aquila* from the Languedocian coast.****Sl. 3: Razmerja med gonosomatskim indeksom (GSI) in širino diska (DW) pri samcih in samicah vrste *M. aquila*, ujetih v obrežnih vodah pokrajine Languedoc.**

Seventeen adult males were collected, 2 in March, 4 in May and 11 between August and October (Tab. 1).

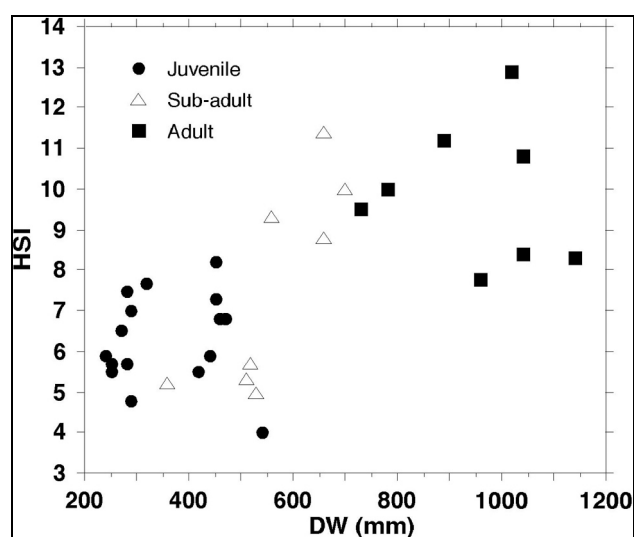
Juvenile females ranged between 240 and 470 mm DW and weighing between 190 and 1,405 g. Sixteen juveniles were collected, fourteen from August to October, one in March and one in May (Tab. 1). Eight sub-adults were captured, 2 specimens in May and 6 between August and October. The smallest sub-adult was 360 mm DW and weighed 862 g; the largest specimen was 700 mm DW and weighed 4,850 g. Eight adults were collected, a single specimen in May and seven between August and October (Tab. 1). The smallest adult was 730 mm DW and weighed 6,100 g; the largest was 1,140 mm and weighed 29,400 g.

Considering the whole sample, values of male HSI (Fig. 2), did not significantly differ from those of female HSI ( $F = 0.06$ ,  $df = 1$ ,  $p = 0.94$ ). Similar patterns were recorded in GSI values (Fig. 3) between males and females ( $F = 3.53$ ,  $df = 1$ ,  $p = 0.75$ ). The highest value of HSI in males was recorded in the smallest free-swimming specimen having 210 mm DW, and decreased from DW of about 250 mm DW onward (Fig. 4). HSI then globally increased; when males entered maturation stage and became sub-adults, HSI reached the highest values in adult specimens. Among males, however, no significant differences were detected in HSI values between juveniles and sub-adults ( $df = 2$ ,  $p = 0.423$ ) and between sub-adults and adults ( $df = 2$ ,  $p = 0.178$ ). By contrast, HSI



**Fig. 4:** Variations in hepatosomatic index (HSI) vs disc width (DW) in juvenile, sub-adult and adult males of *M. aquila* from the Languedocian coast.

**Sl. 4:** Razlike med hepatosomatskim indeksom (HSI) in širino diska (DW) pri mladostnih, skoraj odraslih in odraslih samcih vrste *M. aquila*, ujetih v obrežnih vodah pokrajine Languedoc.



**Fig. 5:** Variations in hepatosomatic index (HSI) vs disc width (DW) in juvenile, sub-adult and adult females of *M. aquila* from the Languedocian coast.

**Sl. 5:** Razlike med hepatosomatskim indeksom (HSI) in širino diska (DW) pri mladostnih, skoraj odraslih in odraslih samcih vrste *M. aquila*, ujetih v obrežnih vodah pokrajine Languedoc.

showed significant differences in females (Fig. 5), between juveniles and sub-adults ( $df = 2$ ,  $p = 0.043$ ) and between sub-adults and adults ( $df = 2$ ,  $p = 0.030$ ).

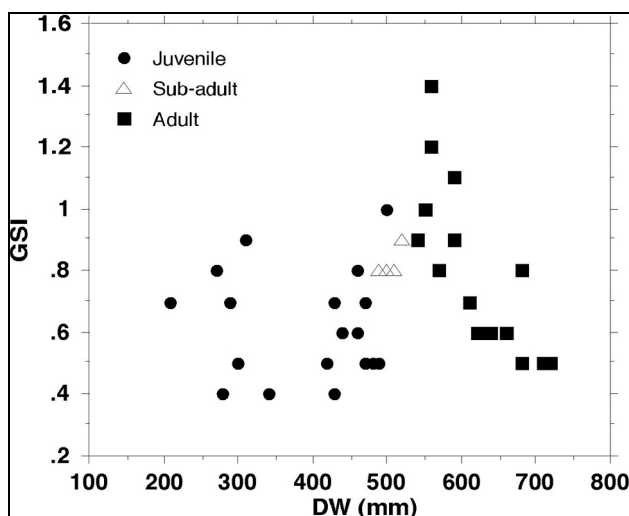
The male GSI values significantly increased with TL of specimens (Fig. 6); additionally, they showed significant differences between juveniles and sub-adults ( $df = 2$ ,  $p = 0.010$ ), and between sub-adults and adults ( $df = 2$ ,  $p = 0.040$ ). Similar patterns were observed in female GSI values (Fig. 7); however, significant differences in GSI values were recorded between juvenile and sub-adult specimens ( $df = 2$ ,  $p = 0.025$ ), and between sub-adults and adults ( $df = 2$ ,  $p = 0.047$ ).

## DISCUSSION

Previously, Capapé *et al.* (2007) showed that both male and female eagle rays from the Languedocian coast demonstrated a positive relationship between disc width (DW) and total mass (TM), and disc width (DW) and liver mass (LM). This relationship, however, significantly differed between males and females, for it was significantly higher in the former than in the latter. The high HSI values observed in both males and females could be explained by the role of liver in buoyancy in agreement with previous reports (Bones & Robert, 1969; Baldrige Jr., 1970, 1972; Rossouw, 1987). Additionally, these significant differences between males and females (Capapé *et al.*, 2008), suggested that liver plays an important role in the life cycle of the latter (Oddone & Velasco, 2006). Liver size is sexually dimorphic in both

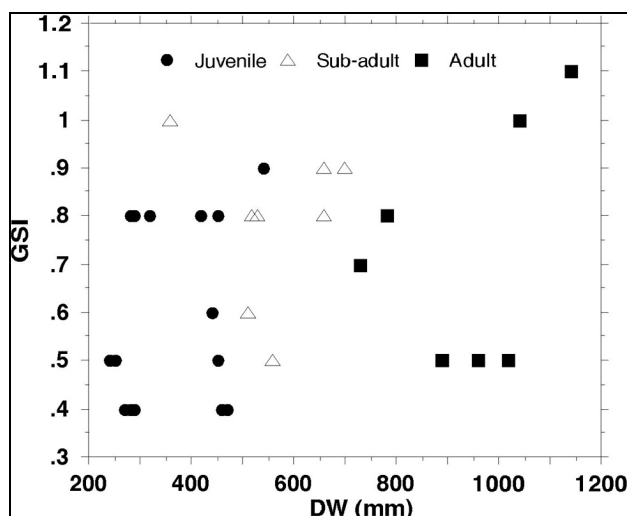
oviparous and viviparous chondrichthyan species. A larger liver may allow females to maximize the production of yolk, such as in the viviparous lesser guitarfish *Rhinobatos annulatus* Müller & Henle, 1841 (Rossouw, 1987), as well as in the small spotted catshark (García-Garrido *et al.*, 1990; Capapé *et al.*, 2008) and the small-nose fanskate *Sympterygia bonapartii* (Magrabaña *et al.*, 2002). Moreover, cartilaginous fish store energy as lipids in the liver (Craik, 1978). In viviparous females, larger liver observed may be related to the increased energy expenditure during vitellogenesis, oocyte maturation and gestation. Furthermore, females store large quantities of lipids in the liver during the reproductive cycle (Lucifora *et al.*, 2005). The highest HSI value was recorded in a neonate male and suggested that liver constituted a reserve of nutriment for free-swimming specimens soon after parturition. This hypothesis was corroborated by decreasing HSI values in larger juveniles.

Both HSI and GSI values are lower in *M. aquila* than in other aplacental viviparous species, such as the angular rough shark *Oxynotus centrina* (Linnaeus, 1758), especially in females. The former is a matrotrophic species (*sensu* Hamlett *et al.*, 2005), in which the mother supplements yolk from other sources such as uterine secretions; the phenomenon was called histotrophy by Hamlett *et al.* (2005). The latter is a pure lecithotrophic species (*sensu* Hamlett *et al.*, 2005), the female produces larger and heavier eggs; the mother only protects the embryonic development and supplies inorganic nu-



**Fig. 6:** Variations in gonosomatic index (GSI) vs disc width (DW) in juvenile, sub-adult and adult males of *M. aquila* from the Languedocian coast.

**Sl. 6:** Razlike med gonosomatskim indeksom (GSI) in širino diska (DW) pri mladostnih, skoraj odraslih in odraslih samcih vrste *M. aquila*, ujetih v obrežnih vodah pokrajine Languedoc.



**Fig. 7:** Variations in gonosomatic index (GSI) vs disc width (DW) in juvenile, sub-adult and adult females of *M. aquila* from the Languedocian coast.

**Sl. 7:** Razlike med gonosomatskim indeksom (GSI) in širino diska (DW) pri mladostnih, skoraj odraslih in odraslih samicah vrste *M. aquila*, ujetih v obrežnih vodah pokrajine Languedoc.

triments especially in squatinids, displaying an utero-cloacal gestation (Capapé *et al.*, 2005). Additionally, transfers of nutriment are less evident in *M. aquila* than in *O. centrina*, and similar patterns were observed in an

oviparous species, the smallspotted catshark *Scyliorhinus canicula* (Linnaeus, 1758) from off the Languedocian coast (Capapé *et al.*, 2008).

## NOVI BIOLOŠKI PODATKI O NAVADNEM MORSKEM GOLOBU *MYLIOBATIS AQUILA* (CHONDRICHTHYES: MYLIOBATIDAE) V VODAH POKRAJINE LANGUEDOC (JUŽNA FRANCIJA, SEVERNO SREDOZEMLJE)

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### POVZETEK

Avtorji članka so med raziskavami, ki so jih opravili v vodah južnofrancoske pokrajine Languedoc, ujeli 37 osebkov navadnega morskega goloba *Myliobatis aquila* (Linnaeus, 1758) in zbrali podatke o hepatosomatskem (HIS) in gonosomatskem indeksu (GSI) tako pri samcih kot samicah te vrste. Razmerja med širino diska (DW) in HIS ter med DW in GSI niso pokazala kakšnih pomembnejših razlik med spoloma. Pa vendar so bile najvišje vrednosti HIS in GSI zabeležene tako pri skoraj odraslih kot odraslih osebkih, še posebno pri odraslih samicah, kar kaže na vlogo jeter pri razvoju spolnih žlez in tudi plavnosti.

**Ključne besede:** Chondrichthyes, Myliobatidae, *Myliobatis aquila*, jetra, spolne žleze, Languedoc, Sredozemlje

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## UTILIZATION OF EMPTY HOLES BY TWO ADRIATIC ENDOLITHIC BLENNIES UNDER EXPERIMENTAL CONDITIONS – PRELIMINARY RESULTS

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### ABSTRACT

The present research aimed at studying the relationships between dominance rank and access to holes in two captive blennioid species (*Parablennius incognitus* and *Parablennius zvonimiri*) competing for territory. The fish preference for hole length, diameter and inclination was assessed. The specimens used in the experiments were caught in the Gulf of Trieste by SCUBA diving. Two different types of experiments were performed with three subsamples each: (a) the number of test males was higher than the number of available holes, and (b) fish number was lower than the number of holes. Moreover, each experiment was also separately performed with specimens of *P. zvonimiri* and *P. incognitus* and in a third set with both species together. The results indicate that *P. zvonimiri* is the dominant species, with a strong positive correlation between the dominance ranks and the access to shelters.

**Key words:** *Parablennius zvonimiri*, *Parablennius incognitus*, experimental conditions, holes availability, intra- and interspecific competition, northern Adriatic Sea

## USO DI FORI VUOTI IN DUE SPECIE ENDOLITICHE DI BLENNIDI ADRIATICI IN CATTIVITÀ – RISULTATI PRELIMINARI

### SINTESI

La presente ricerca è stata mirata allo studio delle relazioni tra il rango di dominanza e l'accesso ai fori in due specie di blennidi tenute in cattività (*Parablennius incognitus* e *Parablennius zvonimiri*) ed in competizione per il territorio. È stata inoltre valutata la preferenza dei pesci per lunghezza, diametro ed inclinazione dei fori. Gli individui usati negli esperimenti sono stati catturati nel Golfo di Trieste con tecniche subacquee. Sono stati eseguiti due tipi di esperimenti: (a) numero di maschi superiore al numero di fori disponibili, e (b) numero di maschi inferiore al numero di fori disponibili. Ogni esperimento è stato ripetuto separatamente con individui di *P. zvonimiri*, di *P. incognitus* e una terza serie con individui di entrambe le specie contemporaneamente. I risultati evidenziano che *P. zvonimiri* è la specie dominante, con una forte correlazione positiva fra il rango di dominanza e l'accesso ai fori.

**Parole chiave:** *Parablennius zvonimiri*, *Parablennius incognitus*, esperimenti, disponibilità di fori, competizione intra- ed interspecifica, Adriatico settentrionale

## INTRODUCTION

Males of most intertidal and subtidal fish species establish their breeding territories, and due to the abundance of fishes in this depth range the competition for space is assumed to be high. The majority of blennies (family Blenniidae) are considered to be residents of rocky habitats, since after the larval stage, they live primarily on rocky habitats and spawn there (Gibson, 1982). Members of this family also exhibit male parental care, especially egg guarding. These males show common courtship behaviour of signalling the nest and leading the female to the entrance of the nesting cavities (Almada & Santos, 1995). The Shy blenny *Parablennius incognitus* (Bath, 1968) and the Zvonimir's blenny *Parablennius zvonimiri* (Kolombatović, 1892) are thought to be very closely related (Bath, 1976; Zander, 1986). In the Gulf of Trieste, these two species showed to be partially syntopic (Orlando-Bonaca & Lipej, 2007), so their existence in the same habitat offers the opportunity to study their interactions and competition for the access to shelters.

*P. incognitus* is very common in the rocky sublittoral Mediterranean and Adriatic Sea, as well (Goldschmid & Kotschal, 1981; Kotschal & Goldschmid, 1981; Patzner, 1985; Kotschal, 1988; Illich & Kotschal, 1990; Orlando-Bonaca & Lipej, 2005). In Slovenian coastal waters, it is widespread and it was defined as the least selective blennioid species in terms of microhabitat choice (Orlando-Bonaca & Lipej, 2007; Lipej *et al.*, 2008). It was found nesting mostly in empty date mussel's (*Lithophaga lithophaga*) holes, and occasionally in *Gastrochaena dubia* holes, empty oyster's shells, crevices and in plastic tubes, from the water surface down to 4 meters of depth (Orlando-Bonaca & Lipej, 2008). In Slovenian coastal waters, the cryptobenthic blenny *P. zvonimiri* (*sensu* Miller, 1979, 1996), defined also as a photophobic species (Pallaoro, 1989), showed a strong preference for rocks covered by precoralligenous bioformations, in a depth range from 3 to 10 m, where it is nesting mostly in empty date mussel's holes (Orlando-Bonaca & Lipej, 2008). Endolithic bivalves bore holes that are optimal shelters for breeding, with relatively small entrances, but wide enough interiors for spawning and guarding eggs (Kotschal, 1988).

In the past, *P. zvonimiri* was confused with *P. incognitus* (Segantin, 1968, since both species have similar body length, shape of the head and supra-orbital tentacles, and can exhibit similar red basic colouration. The presence of *P. zvonimiri* in Slovenian coastal waters was confirmed only recently (Lipej *et al.*, 2005), but had previously been known for the North Adriatic (Patzner, 1985; Kotschal, 1988). *P. incognitus* co-occurs in the same habitat with *P. zvonimiri*, since it is a more opportunistic species (Koppel, 1988).

There is a considerable amount of literature concerning the territorial behaviour of blennioids (Stephens *et al.*, 1970; Wirtz, 1978; Nursall, 1981; Almada *et al.*, 1983, 1992, 1994; Gonçalves & Almada, 1998; Gonçalves *et al.*, 2000). However, to our knowledge, only two attempts were made in the past to investigate the spatial resource partitioning among Mediterranean blennies *ex situ* (Koppel, 1988; Faria & Almada, 2001), while another attempt is known from Mexico (Lindquist, 1985). *P. incognitus* was one of the species studied under experimental conditions by Koppel (1988), while for *P. zvonimiri* this is the first experimental investigation of holes utilization by this species.

The aim of this work was to study the relationships between size related dominance rank and access to holes (shelters and nesting sites) in captivity groups of *P. incognitus* and *P. zvonimiri*, species preferences for holes characteristics, as well as intra- and interspecific competition in utilization of holes.

## MATERIAL AND METHODS

Populations of *P. incognitus* and *P. zvonimiri* were observed in their natural habitat in Slovenian coastal waters (Gulf of Trieste, northern Adriatic Sea) by SCUBA diving, during the spring-summer period from 2003 to 2005.

Six males of each species (*P. incognitus* and *P. zvonimiri*) were captured in July 2005 in the Cape Madonna Nature Monument near Piran (Slovenia), at a depth between 3 and 5 m. All specimens were residents, egg-guarding males thriving in holes and were caught with the method proposed by Kotschal (1988). The opening of a plastic bag was held tightly around a selected hole. The male was then disturbed with a tight wand contained in the bag. The fish darted out of its hole and was caught in the bag. Total length as well as head width and height of the evicted fish were measured (Tab. 1) while confined and stretched out within the bag. The fish were then transported to the biological laboratory of the Marine Biology Station (National Institute of Biology) in Piran.

Two aquaria were used, both 80 litres of capacity. In the first, specimens were contained that were not used in the experiment at that moment, while in the second the experiment was carried out. Water temperature in the aquaria ranged from 18 to 23°C. A light-dark regime of 12 h was maintained. The aquarium under observation contained only two air-stones connected with air pumps and one experimental block of siporex with different types of holes. The siporex was selected as it is a soft material to drill. After boring the holes, the siporex block was well washed off before placed in the experimental aquarium. Since the siporex is very light, it was weighted with cryptic weights.

Tab. 1: Measurements of the blenny males used in experiments.

Tab. 1: Meritve samčkov babic, ki so bili uporabljeni v poskusih.

Species	Parameter		
	Total length (mm)	Head width (mm)	Head height (mm)
<i>P. zvonimiri</i>	67	8	11
	55	8	10
	49	7	9
	49	7	9
	48	7	9
	46	6	8
	45	6	8
<i>P. incognitus</i>	57	8	10
	54	7	9
	50	6	8
	44	5	7
	40	5	7
	32	4	6
	30	4	6

Tab. 2: List of experiments with all variables in each experiment.

Tab. 2: Preglednica poskusov z vsemi spremenljivkami v posameznih poskusih.

Experiment	Species	No. specimens	Observation time (h)	Holes			
				No.	Length (cm)	Diameter (mm)	Inclination (°)
1a	<i>P. zvonimiri</i>	6	8	1	5	16	90
				1	7		
				1	9		
				1	12		
1b	<i>P. incognitus</i>	6	8	1	5	16	90
				1	7		
				1	9		
				1	12		
1c	<i>P. zvonimiri</i>	3	8	1	5	16	90
	<i>P. incognitus</i>	3		1	7		
				1	9		
				1	12		
2a	<i>P. zvonimiri</i>	4	12	6	5	16	90
				6	7		
				6	9		
				6	12		
2b	<i>P. incognitus</i>	4	12	6	5	16	90
				6	7		
				6	9		
				6	12		
2c	<i>P. zvonimiri</i>	3	12	6	5	16	90
	<i>P. incognitus</i>	3		6	7		
				6	9		
				6	12		
3a	<i>P. zvonimiri</i>	6	8	1	12	8	90
				1		10	
				1		12	
				1		16	
				1		18	

Experiment	Species	No. specimens	Observation time (h)	Holes			
				No.	Length (cm)	Diameter (mm)	Inclination (°)
3b	<i>P. incognitus</i>	6	8	1	12	8	90
				1		10	
				1		12	
				1		16	
				1		18	
3c	<i>P. zvonimiri</i>	3	8	1	12	8	90
	<i>P. incognitus</i>	3		1		10	
				1		12	
				1		16	
				1		18	
4a	<i>P. zvonimiri</i>	4	12	5	12	18	90
				5		10	
				5		12	
				5		16	
				5		18	
4b	<i>P. incognitus</i>	4	12	5	12	8	90
				5		10	
				5		12	
				5		16	
				5		18	
4c	<i>P. zvonimiri</i>	3	12	5	12	8	90
	<i>P. incognitus</i>	3		5		10	
				5		12	
				5		16	
				5		18	
5a	<i>P. zvonimiri</i>	6	8	1	7	16	0
				1			45
				1			90
				1			135
5b	<i>P. incognitus</i>	6	8	1	7	16	0
				1			45
				1			90
				1			135
5c	<i>P. zvonimiri</i>	3	8	1	7	16	0
	<i>P. incognitus</i>	3		1			45
				1			90
				1			135
6a	<i>P. zvonimiri</i>	4	12	5	7	16	0
				5			45
				5			90
				5			135
6b	<i>P. incognitus</i>	4	12	5	7	16	0
				5			45
				5			90
				5			135
6c	<i>P. zvonimiri</i>	3	12	5	7	16	0
	<i>P. incognitus</i>	3		5			45
				5			90
				5			135

Each experiment started after 16 hours of acclimatization of the involved specimens to the new aquarium. During the experiment, the time that each fish spent in each hole was noted down, as well as the frequency of agonistic encounters among fishes (inter- and intra-specific).

In order to assess the fish preference for hole length, diameter and inclination, two different types of experiments were performed: firstly, in the experimental aquarium the number of fishes was higher than the number of holes, and secondly the number of fishes was smaller than the number of empty holes. Moreover, each experiment was repeated three times: firstly only with specimens of *P. zvonimiri*, secondly with specimens of *P. incognitus*, and thirdly with specimens of both species.

For those experiments, where the number of holes was higher than the number of fishes, the maximum time of hole occupancy was calculated by multiplying the observation time by the number of involved blennies (following Koppel, 1988). For those experiments, where the number of fishes was higher than the number of holes, the maximum time of hole occupancy was calculated by multiplying the observation time by the number of holes. The total time that specimens spent in holes is the sum of the time that each blenny spent in holes.

Six experiments with eighteen sub-experiments were performed (Tab. 2). The angles of inclination of the hole were defined following Koppel (1988): vertical holes – 0°, horizontal holes – 90°, downwards from entrance – 45° and upwards from entrance – 135°.

#### Assessment of the preference for hole length

Experiment 1a, 1b, 1c. In the aquarium, the number of fishes was higher than the number of holes. In a siporex block, 4 holes were drilled with the same diameter (16 mm) and different lengths (5, 7, 9 and 12 cm). All holes were horizontal (90°) and with the same height of the substrate (6 cm). The experiment had three subsamples: 1a – with 6 *P. zvonimiri*, 1b – with 6 *P. incognitus*, 1c – with 3 *P. zvonimiri* and 3 *P. incognitus*. The time of each experiment was two days; observations 4 hours per day. The maximum time of hole occupancy was = 480 min x 4 holes = 1920 min.

Experiment 2a, 2b, 2c. In the aquarium, the number of fishes was lower than the number of holes. In a siporex block, 24 holes were randomly drilled, with the same diameter (16 mm) and different lengths (six holes of 5 cm, six of 7 cm, six of 9 cm and six of 12 cm). All holes were horizontal (90°). The experiment had three subsamples: 2a – with 4 *P. zvonimiri*, 2b – with 4 *P. incognitus*, 2c – with 3 *P. zvonimiri* and 3 *P. incognitus*. The time of each experiment was three days; observations 4 hours per day. The maximum time of hole occupancy for 2a and 2b was = 720 min x 4 blennies = 2880 min, while for 2c was = 720 min x 6 blennies = 4320 min.

#### Assessment of the preference for hole diameter

Experiment 3a, 3b, 3c. In the aquarium, the number of fishes was higher than the number of holes. In a siporex block, 5 holes were drilled with the same length (12 cm) and different diameters (8, 10, 12, 16, 18 mm). All holes were horizontal (90°) and with the same height of the substrate (6 cm). The experiment had three subsamples: 3a – with 6 *P. zvonimiri*, 3b – with 6 *P. incognitus*, 3c – with 3 *P. zvonimiri* and 3 *P. incognitus*. The time of each experiment was two days; observations 4 hours per day. The maximum time of hole occupancy was = 480 min x 5 holes = 2400 min.

Experiment 4a, 4b, 4c. In the aquarium, the number of fishes was lower than the number of holes. In a siporex block, 25 holes were randomly drilled with the same length (12 cm) and different diameters (five holes of 8 mm, five of 10 mm, five of 12 mm, five of 16 mm, five of 18 mm). All holes were horizontal (90°). The experiment had three subsamples: 4a – with 4 *P. zvonimiri*, 4b – with 4 *P. incognitus*, 4c – with 3 *P. zvonimiri* and 3 *P. incognitus*. The time of each experiment was three days; observations 4 hours per day. The maximum time of hole occupancy for 4a and 4b was = 720 min x 4 blennies = 2880 min, while for 4c was = 720 min x 6 blennies = 4320 min.

#### Assessment of the preference for hole inclination

Experiment 5a, 5b, 5c. In the aquarium, the number of fishes was higher than the number of holes. In a siporex block, 4 holes were drilled with the same length (7 cm) and the same diameter (16 mm), with different inclination (0°, 45°, 90° and 135°). The experiment had three subsamples: 5a – with 6 *P. zvonimiri*, 5b – with 6 *P. incognitus*, 5c – with 3 *P. zvonimiri* and 3 *P. incognitus*. The time of each experiment was two days; observations 4 hours per day. The maximum time of hole occupancy was = 480 min x 4 holes = 1920 min.

Experiment 6a, 6b, 6c. In the aquarium, the number of fishes was lower than the number of holes. In a siporex block, 20 holes were randomly drilled, with the same length (7 cm) and the same diameter (16 mm), with different inclination (five holes of 0°, five of 45°, five of 90° and five of 135°). The experiment had three subsamples: 6a – with 4 *P. zvonimiri*, 6b – with 4 *P. incognitus*, 6c – with 3 *P. zvonimiri* and 3 *P. incognitus*. The time of each experiment was three days; observations 4 hours per day. The maximum time of hole occupancy for 6a and 6b was = 720 min x 4 blennies = 2880 min, while for 6c was = 720 min x 6 blennies = 4320 min.

The time spent in holes in the first series of experiments (1, 3, 5) with more fish than holes is the result of intra- (a, b) and interspecific (c) competition and size related dominance in utilization of holes, influenced by possible interactions of these factors. The second series

of experiments (2, 4, 6) with more holes than fish is mostly answering the question on preferences for holes characteristics by size or species.

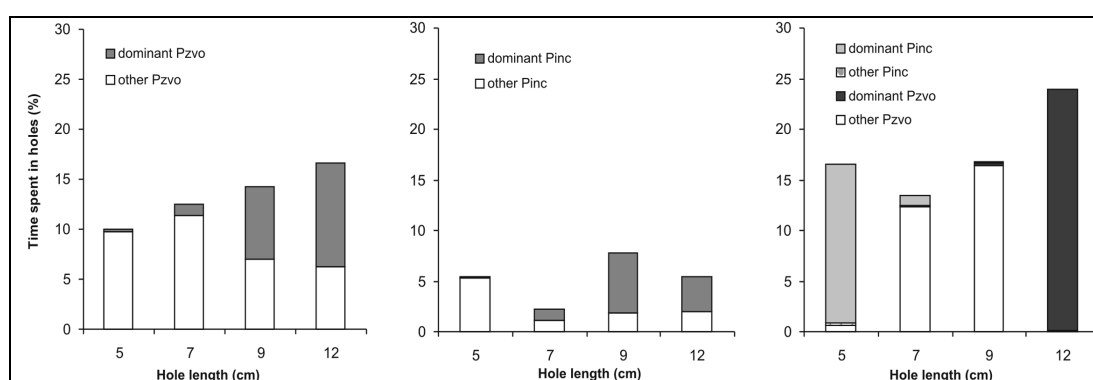
## RESULTS

### Assessment of the preference for hole length

During experiment 1a, the largest specimen among six *P. zvonimiri* showed to be the dominant male in the aquarium (Tab. 2). It occupied mostly 12 cm and 9 cm long holes (Fig. 1), while smaller males occupied other empty holes. The five subordinate males spent most of the time in the 7 cm long hole. Agonistic encounters for

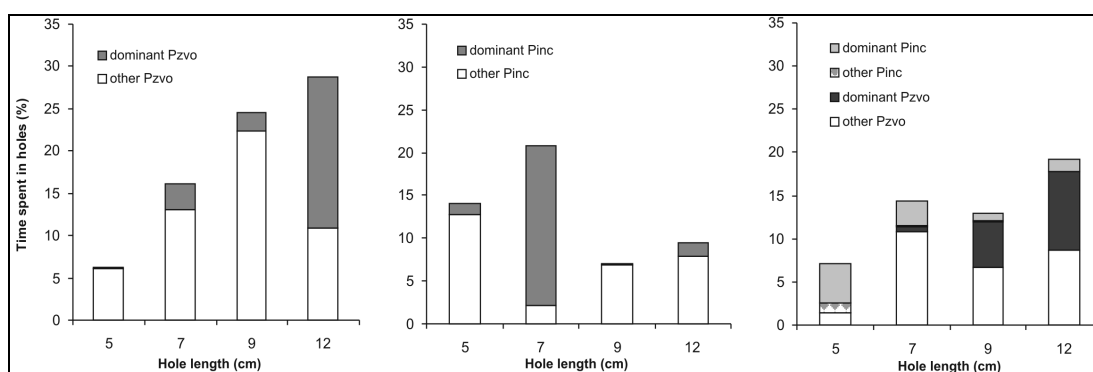
the possession of holes occurred during the entire observation time. Jointly, the six males spent in holes 53.4% of the maximum time of hole occupancy.

During experiment 1b, the largest specimen among six *P. incognitus* showed to be the dominant male in the aquarium. It occupied mostly the 9 cm long hole (Fig. 1), while the five subordinate males spent most of the time in the 5 cm long hole. Agonistic encounters for the possession of holes occurred during the entire observation time and the males spent more time in agonistic encounter in front of the holes than in holes. Jointly, the six males spent in holes 20.9% of the maximum time of hole occupancy.



**Fig. 1:** Settling experiments performed with *P. zvonimiri* and *P. incognitus* under laboratory conditions with 4 holes of varying lengths. Hole diameter: 16 mm; hole inclination: 90° (horizontal). Experiments: 1a: with 6 *P. zvonimiri*, 1b: with 6 *P. incognitus*, 1c: with 3 *P. zvonimiri* and 3 *P. incognitus*.

**Sl. 1:** Poskusi s *P. zvonimiri* in *P. incognitus* v eksperimentalnih razmerah, s 4 rovi različnih dolžin. Premer rorov: 16 mm; naklon rorov: 90° (horizontalni). Poskusi: 1a: s 6 *P. zvonimiri*, 1b: s 6 *P. incognitus*, 1c: s 3 *P. zvonimiri* in 3 *P. incognitus*.



**Fig. 2:** Settling experiments performed with *P. zvonimiri* and *P. incognitus* under laboratory conditions with 24 holes of varying lengths (six holes of 5 cm, six of 7 cm, six of 9 cm and six of 12 cm). Hole diameter: 16 mm; hole inclination: 90° (horizontal). Experiments: 2a: with 4 *P. zvonimiri*, 2b: with 4 *P. incognitus*, 2c: with 3 *P. zvonimiri* and 3 *P. incognitus*.

**Sl. 2:** Poskusi s *P. zvonimiri* in *P. incognitus* v eksperimentalnih razmerah, s 24 rovi različnih dolžin (šest rorov 5 cm, šest rorov 7 cm, šest rorov 9 cm, šest rorov 12 cm). Premer rorov: 16 mm; naklon rorov: 90° (horizontalni). Poskusi: 2a: s 4 *P. zvonimiri*, 2b: s 4 *P. incognitus*, 2c: s 3 *P. zvonimiri* in 3 *P. incognitus*.

During experiment 1c, *P. zvonimiri* showed to be the dominant species, and the larger specimen of *P. zvonimiri* was the dominant male in the aquarium. Males of *P. zvonimiri* occupied the three longer holes (Fig. 1), while the dominant male of *P. incognitus* occupied the 5 cm long hole. Agonistic encounters for the possession of holes occurred during the entire observation time. Jointly, the three *P. zvonimiri* males spent in holes 53.8% of the maximum time of hole occupancy, the three *P. incognitus* males only 17.1%.

During experiment 2a (Tab. 2), the dominant male of *P. zvonimiri* occupied mostly 12 cm long holes (Fig. 2), while smaller males showed a preference for 9 cm holes. The number of agonistic encounters for the possession of holes was negligible. Jointly, the four males spent in holes 75.7% of the maximum time of hole occupancy. They spent almost all the time (96.5%) in holes in the lower half of the experimental block.

During experiment 2b, the dominant male of *P. incognitus* occupied mostly 7 cm long holes (Fig. 2), while smaller males showed a preference for 5 cm holes. Despite the high number of empty holes, the dominant male continuously dislodged subordinates from each hole it visited. Jointly the four males spent in holes 51.5% of the maximum time of hole occupancy; 93.3% of that in holes in the lower half of the experimental block.

During experiment 2c, the dominant *P. zvonimiri* mostly occupied 12 cm long holes, while the other two specimens of this species spent more time in 7 cm long holes (Fig. 2). The dominant male of *P. incognitus* mostly occupied 5 cm long hole. Jointly, the three *P. zvonimiri* males spent in holes 42.5% of the maximum time of hole occupancy (96.2% of that in holes in the lower half of the experimental block), the three *P. incognitus* males only 11.1% (91.1% in the lower half of the block).

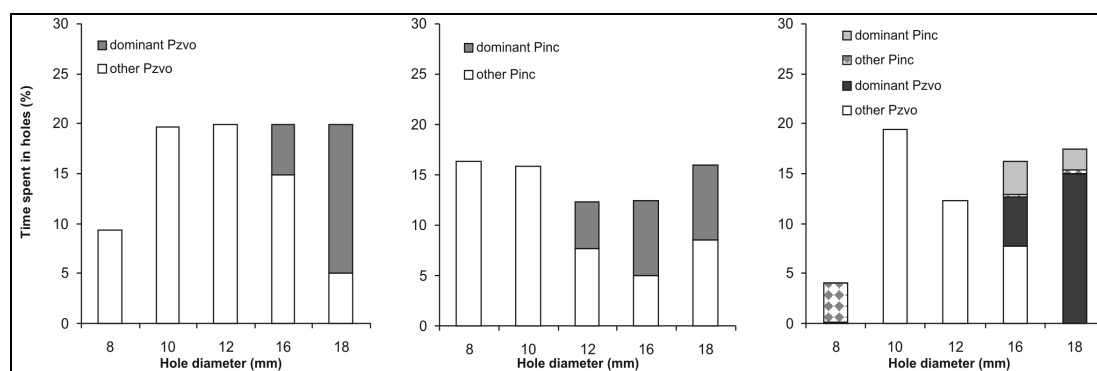
### Assessment of the preference for hole diameter

During experiment 3a (Tab. 2), the dominant male of *P. zvonimiri* occupied mostly the hole with 18 mm of diameter (Fig. 3), while smaller males showed a preference for 10 and 12 mm large holes. Agonistic encounters for the possession of holes occurred during the entire observation time. Jointly, males spent in holes 88.7% of the maximum time of hole occupancy.

During experiment 3b, the dominant *P. incognitus* occupied mostly holes with 16 and 18 mm of diameter (Fig. 3), while the five subordinate males spent most of the time in holes 8 and 10 mm large. Agonistic encounters for the possession of holes occurred during the entire observation time. Jointly, males spent in holes 72.8% of the maximum time of hole occupancy.

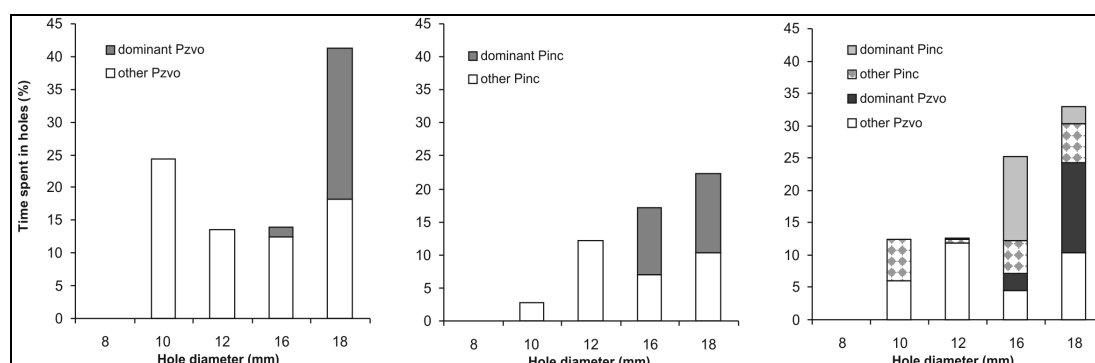
During experiment 3c, the dominant *P. zvonimiri* occupied mostly the hole with 18 mm of diameter, while the two subordinates of this species occupied mostly the 10 mm large hole (Fig. 3). Dominant males of both species fought for the possession of 16 and 18 mm large holes. The two smaller specimens of *P. incognitus* occupied the 8 mm large hole. Agonistic encounters for the possession of holes occurred during the whole observation time. Jointly, *P. zvonimiri* males spent in holes 59.5% of the maximum time of hole occupancy, *P. incognitus* males only 10.1%.

During experiment 4a (Tab. 2), the dominant male of *P. zvonimiri* occupied mostly 18 mm large holes (Fig. 4). Smaller males showed a preference for 10 mm large holes, avoiding completely the narrowest holes of 8 mm. There were no agonistic encounters for the possession of holes. Jointly, males spent in holes 93.2% of the maximum time of hole occupancy (94.7% in holes in the lower half of the experimental block).



**Fig. 3:** Settling experiments performed with *P. zvonimiri* and *P. incognitus* under laboratory conditions with 5 holes of varying diameter. Hole length: 12 cm; hole inclination: 90° (horizontal). Experiments 3a: with 6 *P. zvonimiri*, 3b: with 6 *P. incognitus*, 3c: with 3 *P. zvonimiri* and 3 *P. incognitus*.

**Sl. 3:** Poskusi s *P. zvonimiri* in *P. incognitus* v eksperimentalnih razmerah, s 5 rovi različnih premerov. Dolžina ro-vov: 12 cm; naklon rovv: 90° (horizontalni). Poskusi: 3a: s 6 *P. zvonimiri*, 3b: s 6 *P. incognitus*, 3c: s 3 *P. zvonimiri* in 3 *P. incognitus*.



**Fig. 4: Settling experiments performed with *P. zvonimiri* and *P. incognitus* under laboratory conditions with 25 holes of varying diameter (five holes of 8 mm, five of 10 mm, five of 12 mm, five of 16 mm, five of 18 mm). Hole length: 12 cm; hole inclination: 90° (horizontal). Experiments: 4a: with 4 *P. zvonimiri*, 4b: with 4 *P. incognitus*, 4c: with 3 *P. zvonimiri* and 3 *P. incognitus*.**

**Sl. 4: Poskusi s *P. zvonimiri* in *P. incognitus* v eksperimentalnih razmerah, s 25 rovi različnih premerov (pet rofov 8 mm, pet rofov 10 mm, pet rofov 12 mm, pet rofov 16 mm, pet rofov 18 mm). Dolžina rofov: 12 cm; naklon rofov: 90° (horizontalni). Poskusi: 4a: s 4 *P. zvonimiri*, 4b: s 4 *P. incognitus*, 4c: s 3 *P. zvonimiri* in 3 *P. incognitus*.**

During experiment 4b, the dominant *P. incognitus* occupied holes with 16 and 18 mm of diameter (Fig. 4), while the others spent most of the time in holes 12 mm large, avoiding completely the narrowest holes of 8 mm. The dominant male was aggressive against subordinates, despite the high number of empty holes. Jointly, males spent in holes 54.3% of the maximum time of hole occupancy (91.6% in holes in the lower half of the experimental block).

During experiment 4c, the dominant *P. zvonimiri* occupied mostly holes with 18 mm of diameter, while the dominant *P. incognitus* persisted mostly in 16 mm large holes (Fig. 4). The dominant *P. zvonimiri* always persecuted the dominant *P. incognitus* when it stopped in the largest holes. The other two *P. zvonimiri* mostly occupied 12 mm large holes. Subordinates *P. incognitus* mostly persisted in holes with 10 mm diameter, avoiding completely the narrowest holes of 8 mm. Jointly *P. zvonimiri* males spent in holes 49.2% of the maximum time of hole occupancy (98.2% of that in holes in the lower half of the experimental block). *P. incognitus* males spent in holes 34.0% of the maximum time (93.9% in the lower half of the block).

#### Assessment of the preference for hole inclination

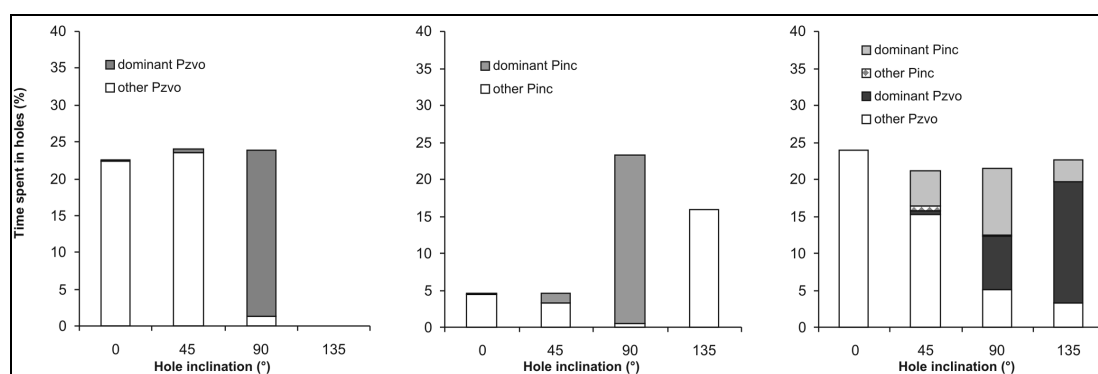
During experiment 5a (Tab. 2), the dominant male of *P. zvonimiri* occupied mostly the horizontal hole (90°) (Fig. 5). All the males avoided the hole with 135° inclination. Subordinate males stopped almost equally in vertical (0°) and sloping upwards holes (45°). Jointly, males spent in holes 70.4% of the maximum time of hole occupancy.

During experiment 5b, the dominant male of *P. incognitus* occupied mostly the horizontal hole (90°) (Fig. 5). Subordinate males spent most of the time in the hole with 135° inclination. Agonistic encounters for the possession of holes occurred during the entire observation time and the males spent more time in agonistic encounter in front of the holes than in holes. Jointly, males spent in holes 48.3% of the maximum time of hole occupancy.

During experiment 5c, the dominant male of *P. zvonimiri* constantly persecuted the dominant male of *P. incognitus* in all the holes occupied by the latter. The dominant male of *P. zvonimiri* stopped mostly at the hole with 135° inclination (Fig. 5), while the dominant male of *P. incognitus* occupied mostly the horizontal hole (90°). The other two males of the latter species fought for the sloping upwards hole (45°). Subordinates of *P. zvonimiri* mostly occupied the vertical hole (0°). Jointly, *P. zvonimiri* males spent in holes 71.7% of the maximum time of hole occupancy, *P. incognitus* males only 17.5%.

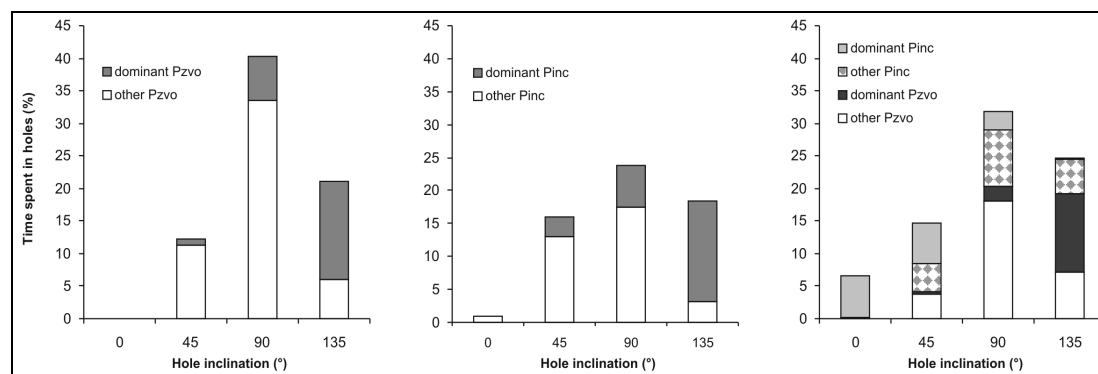
During experiment 6a (Tab. 2), the dominant male of *P. zvonimiri* occupied mostly holes with 135° inclination (Fig. 6), while the subordinates occupied mostly horizontal holes (90°). There were no agonistic encounters for the possession of holes. Jointly, males spent in holes 73.8% of the maximum time of hole occupancy.

During experiment 6b, the dominant male of *P. incognitus* occupied mostly holes with 135° inclination (Fig. 6), while the subordinates occupied mostly horizontal holes (90°). The dominant male dislodged all the others that occupied holes with 135° inclination. Jointly, males spent in holes 59.3% of the maximum time of hole occupancy.



**Fig. 5:** Settling experiments performed with *P. zvonimiri* and *P. incognitus* under laboratory conditions with 4 holes of varying inclination. Hole length: 7 cm; hole diameter: 16 mm. Experiments: 5a: with 6 *P. zvonimiri*, 5b: with 6 *P. incognitus*, 5c: with 3 *P. zvonimiri* and 3 *P. incognitus*.

**Sl. 5:** Poskusi s *P. zvonimiri* in *P. incognitus* v eksperimentalnih razmerah, s 4 rovi različnih naklonov. Dolžina ro-vov: 7 cm; premer ro-vov: 16 mm. Poskusi: 5a: s 6 *P. zvonimiri*, 5b: s 6 *P. incognitus*, 5c: s 3 *P. zvonimiri* in 3 *P. in-cognitus*.



**Fig. 6:** Settling experiments performed with *P. zvonimiri* and *P. incognitus* under laboratory conditions with 20 holes of varying inclination (five holes of 0°, five of 45°, five of 90° and five of 135°). Hole length: 7 cm; hole diameter: 16 mm. Experiments: 6a: with 4 *P. zvonimiri*, 6b: with 4 *P. incognitus*, 6c: with 3 *P. zvonimiri* and 3 *P. incognitus*.

**Sl. 6:** Poskusi s *P. zvonimiri* in *P. incognitus* v eksperimentalnih razmerah, z 20 rovi različnih naklonov (pet ro-vov 0°, pet ro-vov 45°, pet ro-vov 90°, pet ro-vov 135°). Dolžina ro-vov: 7 cm; premer ro-vov: 16 mm. Poskusi: 6a: s 4 *P. zvonimiri*, 6b: s 4 *P. incognitus*, 6c: s 3 *P. zvonimiri* in 3 *P. incognitus*.

During experiment 6c, the dominant *P. zvonimiri* occupied mostly holes with 135° inclination (Fig. 6), while subordinates of this species mostly persisted in horizontal holes (90°). The dominant *P. zvonimiri* always immediately dislodged the dominant *P. incognitus* when it entered horizontal holes and holes with 135° inclination. The dominant *P. incognitus* mostly persisted in vertical holes (0°) and in sloping upwards holes (45°). Subordinates *P. incognitus* mostly occupied horizontal holes (90°). Jointly, *P. zvonimiri* males spent in holes 43.7% of the maximum time of hole occupancy, *P. incognitus* males 34.1%.

## DISCUSSION

The results of the present study attest that between *P. incognitus* and *P. zvonimiri*, the latter is the dominant species where the two coexist. The ecological differences between the two species in the natural environment indicate that *P. zvonimiri* is more specialized of the two blennies regarding habitat choice, since it is found in a smaller number of different microhabitats and has a very high affinity for date mussel holes (Patzner, 1985; Kotschal 1988; Orlando-Bonaca & Lipej, 2007). Thus it defends more aggressively its territory and its nesting place from males of other less specialized species. Moreover, *P. incognitus*, which lives in a higher

number of microhabitats and uses different type of shelters as nesting places, resulted to be less territorial. Also in the experiments performed with two species by Koppel (1988) *L. caneavae*, more specialized than *P. incognitus*, resulted more territorial and dominant throughout the year. Males of *L. caneavae* constantly defend their territories from males of the opportunistic species *P. incognitus*, although they have different feeding habits (Goldschmid & Kotrschal, 1981; Koppel, 1988). Also for three sympatric chaenopsid blennies of the genus *Acanthemblemaria* from Mexico, resulted that the more specialized species has the highest competitive ability for the available shelters (Lindquist, 1985).

For *P. zvonimiri* and *P. incognitus*, the results of this research point out a strong positive correlation between the dominance rank and the access to shelters. The dominant male dislodges subordinates from shelters, and when the number of fishes per aquarium increased (or the number of available holes decreased), the number of agonistic encounters and dislodgements increased, as well. This had previously been noted for other two blennies, *L. caneavae* and *C. galerita* (Koppel, 1988; Faria & Almada, 2001). In *P. zvonimiri* and *P. incognitus*, the dominance rank is positively correlated to the body size of the fish. For both species, the dominant male was the bigger one. During our experiments, dominant males did not restrict their visits only to the selected holes, but they temporarily visited other nearby holes and expelled subordinates from them. When the preferred hole remained vacant, it was temporarily occupied by subordinate males. Dominant males did not limit their access to a single hole, but they dislodged subordinates also from holes they visited less frequently. This observation agrees with the conception of a "diffuse territoriality", proposed by Gibson (1968), where a male would not defend a single shelter, but a network of familiar holes scattered in its home range. Our field experiences (Orlando-Bonaca & Lipej, 2007), however, confirms the findings of Almada *et al.* (1983, 1990): in the natural environment where females are present, the "diffuse territoriality" for breeding males would change to a "traditional territorial defence", where males tend to concentrate their visit to a single hole.

*P. zvonimiri* chose holes that were much longer and larger than its body, which is in agreement with our field observations (Orlando-Bonaca & Lipej, 2008). Since the species did not choose holes that closely matched its body size, we can assume that it is probably less exposed to interspecific competition for holes than the other species. In experiments with two species, *P. incognitus* chose holes that are negligible longer and larger than its body, which could prevent small males from being dislodged by bigger ones. It seems likely that during its membership in a group, each individual acquires information about which individuals are dominants and which shelter it could invade. This observa-

tion is supported also by the results of Faria & Almada (2001). In the natural environment, with avoiding competition for the nesting place, the species provides for itself a more successful reproduction. Like in our field observations, also in the aquarium both species showed a preference for horizontal holes. However, it is interesting to note that the dominant *P. zvonimiri* never entered the hole with 135° inclination during a single species experiment with 4 holes, while it was fighting for the possession of this hole with the dominant *P. incognitus* during the experiment with two species. Probably the two dominant males acquire information about each other during the months passed in aquaria. Both species showed a preference for holes positioned in the lower half of the experimental block, which for *P. zvonimiri* is in accordance with the position of occupied holes in boulders in the natural environment (*own observations*). *P. zvonimiri* were found *in situ* mostly in holes in the shade of boulders and rocks (Orlando-Bonaca & Lipej, 2008).

The number of agonistic encounters and dislodgement was the highest when in the aquaria with more fish than available holes. Although this is true for both species, the dominant *P. incognitus* remained aggressive towards its subordinates even in experiments with less fish than holes, which was not the case of *P. zvonimiri*. Anyhow, since dominant males of both species always won intraspecific fights with subordinates, the preference of these smaller males for hole variables was evident only in experiments with much more holes than fish.

The results suggest that agonistic interactions among males of *P. zvonimiri* and *P. incognitus* play an important role in the control of a network of holes, used as shelters and in the natural environment also as nesting sites. Further experimental work needs to be carried out in order to verify if this conclusion could be confirmed for other Mediterranean blennies living on rocky bottom.

## CONCLUSIONS

Under experimental conditions it was established that between *P. incognitus* and *P. zvonimiri* the latter is the dominant species. Males of *P. incognitus* were always subordinated to males of *P. zvonimiri*. In experiments with both species, *P. incognitus* chose holes that were negligible longer and larger than its body, which could prevent small males from being dislodged by bigger ones. *P. zvonimiri* always chose holes that were much longer and larger than its body. Dominant males did not limit their access to a single hole, but they dislodged subordinates also from holes they visited less frequently. Agonistic interactions among males of *P. zvonimiri* and *P. incognitus* play an important role in the control of a network of holes.

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## UPORABA PRAZNIH ROVOV PRI DVEH VRSTAH JADRANSKIH ENDOLITSKIH BABIC V EKSPERIMENTALNIH RAZMERAH – PRELIMINARNI REZULTATI

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## POVZETEK

Avtorja opisujeta izsledke raziskave o tekmovanju za prostor pri dveh vrstah jadranskih babic (*Parablennius incognitus* in *Parablennius zvonimiri*) v smislu dominance in zasedenosti izbranih rogov. Ocenila sta preference babic za dolžino, premer in naklon rova. Babice so bile ulovljene v slovenskem obalnem morju z uporabo avtonomne potapljaške opreme. Opravila sta dva niza laboratorijskih poskusov s po tremi podvzorci, v katerih je bilo (a) število samcev večje od števila razpoložljivih rogov ali pa (b) je bilo rogov več kot rib. Dva niza poskusov sta bila opravljena z vsako vrsto posebej, v tretjem pa z obema vrstama skupaj.

Na podlagi rezultatov se je izkazalo, da je jelenjeroga babica (*P. zvonimiri*) dominantna vrsta, ki kaže veliko premozorazmerno korelacijo med dominanco in zasedenostjo rogov. V vseh poskusih je jelenjeroga babica izrinila jelenko (*P. incognitus*).

**Ključne besede:** *Parablennius zvonimiri*, *Parablennius incognitus*, eksperimentalne razmere, razpoložljivost rogov, intra- in interspecifično tekmovanje, severni Jadran

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## PRIME VALUTAZIONI DELL'IMPATTO DEL DISTURBO ANTROPICO ALLA RISERVA NATURALE MARINA MIRAMARE: IL MONITORAGGIO DELLA ZONA DI MAREA

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### SINTESI

*Lo studio ha avuto lo scopo di evidenziare e valutare il possibile impatto legato alla fruizione didattica che insiste nella zona più sensibile della Riserva Marina di Miramare (Trieste), la zona di marea. Sono state definite e campionate due stazioni di superficie 50x100 cm, una delle quali è stata isolata e tenuta chiusa alla fruizione, all'interno della spiaggia a sabbia e ciottoli sulla quale si svolgono le attività di educazione ambientale. I dati, raccolti nell'arco di un periodo di 2 anni, sono stati analizzati utilizzando l'analisi della varianza (ANOVA) e i criteri dell'analisi multivariata e i risultati ottenuti hanno evidenziato che nel sito chiuso la comunità mantiene nel tempo una costanza nella macrostruttura, contrariamente al sito di fruizione didattica dove si evidenzia una maggiore variabilità.*

**Parole chiave:** area marina protetta, comunità di marea, impatto antropico, calpestio, nMDS, monitoraggio

## PRELIMINARY EVALUATION OF HUMAN TRAMPLING IMPACT INSIDE THE MIRAMARE MARINE PROTECTED AREA: THE TIDAL ZONE MONITORING

### ABSTRACT

*The study aims at identifying and assessing any impact connected to educational activities carried out in the tidal zone, the most sensitive area in Miramare Natural Marine Reserve (Trieste). Two stations (50x100 cm) were identified and sampled on the sandy and pebbly beach, where environmental education activities are carried out. Admittance has been forbidden in one of them. The analysis of variance (ANOVA) test was used to analyse data and to obtain indication on the tidal zone status, together with multivariate analyses to make a distinction between the trampled and non-trampled areas. Data were gathered over 2 years and results obtained have highlighted that the community is characterised by stable macrostructure inside the closed area, while greater variability is witnessed in the area, where activities are allowed.*

**Key words:** marine protected area, tidal zone community, anthropic impact, trampling, nMDS, monitoring

## INTRODUZIONE

A seguito dell'avvenuta certificazione ambientale EMAS (Reg. CE 761/2001) del Soggetto Gestore della Riserva Marina di Miramare (Trieste), si è avviato un programma di monitoraggio allo scopo di analizzare l'obiettivo ritenuto prioritario di "Conservazione della diversità specifica nella zona di marea". Si è quindi iniziato un programma di studio allo scopo di valutare, qualora ci fosse, l'effetto impattante derivante dalle attività di fruizione didattica nella zona più sensibile della Riserva Marina, la zona di marea.

Il tema della valutazione del disturbo legato alla fruizione è importante perché riferito più in generale ad una strategia di gestione delle aree protette. Parchi e riserve, infatti, devono confrontarsi giornalmente con la necessità di conservare uno spazio senza però precludere totalmente le zone sotto tutela all'accesso del pubblico. Affinché le azioni di gestione siano efficaci occorre una conoscenza delle conseguenze che seguono ad un'attività di fruizione oltre che del sistema sul quale insistono (Jenkins *et al.*, 2002).

Ogni anno la Riserva è frequentata da una media di 7.000 studenti impegnati in attività didattiche diverse alcune delle quali hanno come obiettivo lo studio della zona di mesolitorale su una spiaggia a ciottoli e ghiaia, denominata spiaggia delle ex – Scuderie, situata all'interno dell'area di riserva integrale (Fig. 1) dove i ragazzi sono impegnati nell'osservazione dei popolamenti animali e vegetali. Queste attività comportano, oltre al calpestio con possibile azione meccanica di disturbo di

alcuni organismi sessili, anche lo spostamento di sassi e ciottoli allo scopo di osservare gli organismi che si nascondono negli interstizi (Fig. 2).

La spiaggia, esposta a sud, ha una lunghezza di circa 200 metri ed un'ampiezza media di una decina di metri in condizioni di marea zero (livello medio del mare). E' costituita da un substrato di tipo misto che va dalla sabbia alla ghiaia ai ciottoli di dimensioni variabili fino ad arrivare ai grossi massi calcarei (Fig. 3).

## RASSEGNA DEI LAVORI GIÀ PUBBLICATI

Alcuni lavori sul monitoraggio della zona di marea svolti all'interno della Riserva di Miramare sono stati oggetto di tesi di laurea, tesi di master e tesi di dottorato. In particolare, una prima valutazione delle differenze nei popolamenti dentro e fuori all'area marina protetta allo scopo di testare una metodologia di campionamento per possibili impatti, è stata argomento di una tesi di master in "Valutazione e Gestione delle Risorse Biologiche Marine" presso l'Università di Padova (Polo, 2004). Un monitoraggio dei popolamenti macrozoobentonici inframareali come indicatori di disturbo antropico si trova nella tesi di Tempesta (2005) per il dottorato in "Metodologie di Biomonitoraggio dell'Alterazione Ambientale" presso l'Università di Trieste, mentre uno studio sui metodi di analisi del ricoprimento ad Ulvacee del piano mediolitorale della Riserva di Miramare è stato argomento di una tesi di laurea in Scienze Biologiche (Tagliarolo, 2006) presso l'Università di Trieste.

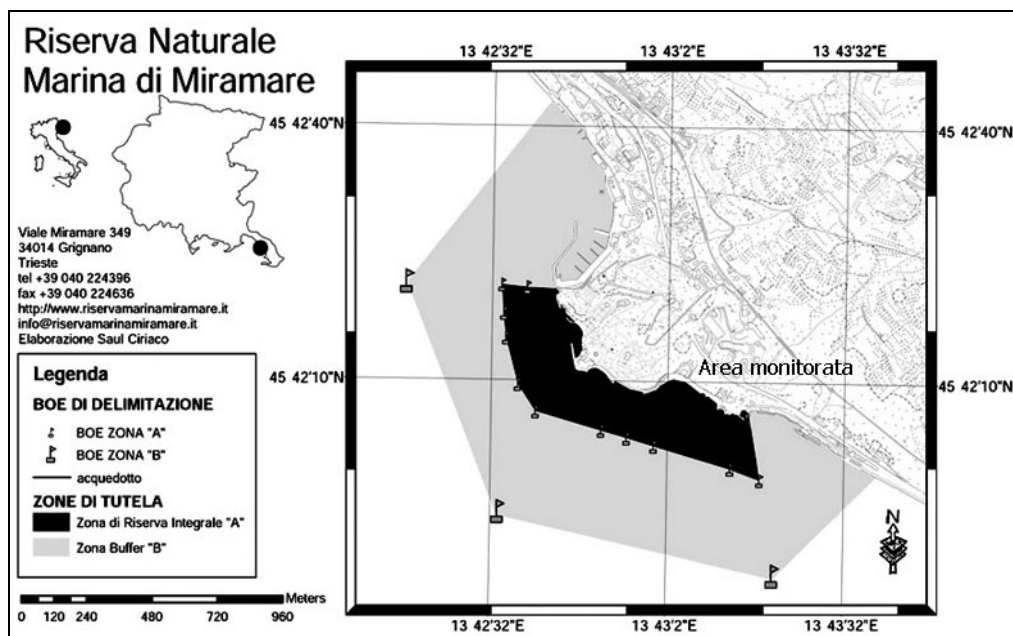
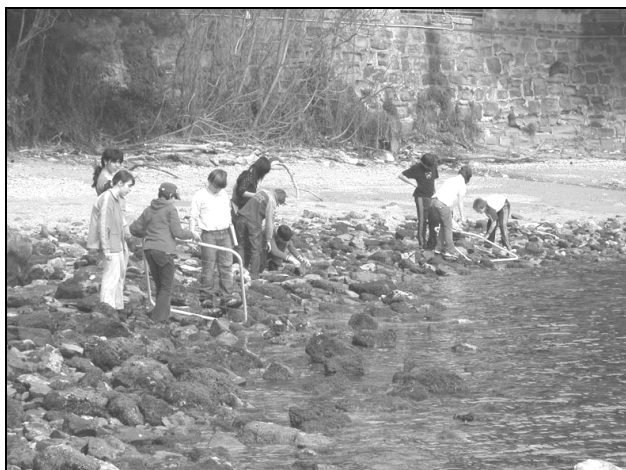


Fig. 1: Localizzazione dell'area di campionamento all'interno della Riserva Marina di Miramare (Trieste).  
Sl. 1: Lokacija vzorčiščnega območja v Morskem rezervatu Miramare (Trst).



**Fig. 2:** Attività di educazione ambientale in zona di marea all'interno della Riserva Marina di Miramare (Trieste).

**Sl. 2:** Izobraževalne dejavnosti v bibavičnem pasu Morskega rezervata Miramare (Trst).



**Fig. 3:** Spiaggia di sabbia e ciottoli, soggetta al calpestio da parte delle scolaresche, in cui si è svolto lo studio.

**Sl. 3:** Od skupin šolarjev poteptano peščeno in prodnato obrežje, kjer je bila opravljena pričujoča študija.

## MATERIALI E METODOLOGIA

Al fine di avviare un programma di valutazione del possibile impatto si è deciso quindi di chiudere alla fruizione una porzione di spiaggia in modo da valutarne le eventuali differenze con il resto della zona calpestata. L'area scelta si trova su una fascia che rimane scoperta con un evento di bassa marea di almeno 40 cm sotto il livello medio del mare. E' infatti questo il limite necessario affinché si scopra una parte di litorale sufficiente allo svolgimento delle attività didattiche. La delimitazione di questo spazio non più accessibile è avvenuta a febbraio 2004 prima quindi dell'inizio delle visite.

Si è avviata così una fase di campionamento della presenza e della abbondanza delle specie su di una superficie campione posizionata in maniera casuale all'interno dell'area chiusa, denominata "Blank", e all'esterno della stessa nell'ambito della parte di spiaggia destinata alle visite, stazione indicata come "Didattica". Il metodo utilizzato è stato quello del plot sampling (Fig. 4) cioè della definizione di un'area di dimensioni note all'interno della quale si identificano, si contano e a volte si misurano gli organismi presenti (Brower *et al.*, 1990).

Su ogni stazione si sono effettuate 8 repliche utilizzando quadrati delle dimensioni di 25x25 cm ciascuno, all'interno dei quali gli organismi animali sono stati contati laddove possibile, mentre per quelle specie sessili che possono avere raggruppamenti di moltissimi individui, si è fatto un conteggio stimato sulla copertura. Le specie coloniali sono state considerate come singoli individui (Davidson *et al.*, 2004). I valori di abbondanza così ottenuti per ogni replica sono stati sommati e mediati in modo da descrivere la stazione "media" Blank e

la stazione "media" Didattica per ogni campionamento. Le repliche sono state in numero di 8 così da raggiungere la superficie minima di campionamento secondo quando indicato dalla curva area/specie (Brower *et al.*, 1990).

La definizione delle dimensioni dell'area di campionamento ha tenuto conto di indicazioni rinvenute in bibliografia: secondo uno studio canadese condotto sulla struttura di un popolamento a mitili nella zona intertidale si riscontra come la variabilità della struttura della comunità sia più evidente su piccola scala mentre aumentando la scala spaziale i campioni risultano essere sempre meno differenziati (McKindsey & Bourget, 2001). Effetti positivi dell'analisi su piccola scala spaziale sono indicati anche da Underwood & Chapman (1996) soprattutto se riferiti a organismi che hanno motilità giornaliera nei piccoli spazi.

I quadrati da campionare sono stati delimitati grazie all'apposizione di picchetti fissi in modo da poter monitorare nel tempo sempre le stesse aree e valutarne così eventuali variazioni.

L'individuazione di solo due zone di studio, una nell'area calpestata e l'altra nella zona chiusa alla fruizione, e quindi l'assenza di ulteriori siti controllo è stata dettata dal tempo limitato in cui era possibile svolgere il campionamento, soggetto al breve periodo in cui le zone rimangono scoperte dall'evento di bassa marea. Il tempo medio di campionamento per coprire una singola stazione (8 repliche) si è aggirato intorno ai 45 minuti / 1 ora. Il tempo totale di campionamento della durata di 2 ore circa, era in alcuni casi il limite massimo di tempo oltre il quale la marea cominciava a risalire arrivando quasi a coprire la fascia da campionare.



**Fig. 4:** Metodo del plot sampling usato per raccogliere dati nelle due aree di studio (calpestata e non calpestata).

**Sl. 4:** Vzorčična metoda, uporabljena za zbiranje podatkov na dveh vzorčičskih območjih (poteptanem in nepoteptanem).

Entrambe le stazioni sono state campionate nello stesso giorno ad eccezione dei casi in cui le condizioni meteorologiche sfavorevoli o eventi di marea ridotti non lo consentissero.

Le stazioni si situano in quella fascia di costa che viene scoperta dagli eventi di bassa marea di almeno 40 cm sotto il livello medio del mare e la zona studiata in queste condizioni è risultata essere composta da ciottoli di lunghezza massima di 30–40 cm e spessore di 10–20 cm alternati ad altri di dimensioni minori, a sassi di grandezza più ridotta e spazi liberi in cui è presente ghiaia (Fig. 4). Sono stati oggetto del conteggio tutti gli organismi rinvenuti sia sopra ai massi e ai ciottoli che sotto di essi che negli interstizi tra la ghiaia e la sabbia.

I campionamenti si sono svolti ad ogni bassa marea "utile" (circa ogni 15 giorni/1 mese) (Colucci *et al.*, 2003, 2004, 2005). In totale sono stati campionati 25 volte i siti Didattica e Blank durante un periodo di 22 mesi, dal 2 agosto 2004 al 17 maggio 2006.

Nello stesso periodo, le giornate in cui si è svolta l'attività didattica con conseguente calpestio sono elencate nella Tabella 1.

#### L'analisi multivariata sulle due aree campione

I dati raccolti sono stati analizzati mediante criteri di analisi multivariata utilizzando il pacchetto di analisi statistica PAST ver. 1.74 (Hammer *et al.*, 2001), disponibile open source dal sito internet dell'Università di Oslo (<http://folk.uio.no/ohammer/past>).

L'analisi è stata svolta allo scopo di mettere a confronto le due aree a diversa fruizione tenendo conto

di tutte le specie rinvenute. Non essendo rispettata la condizione di distribuzione di normalità per tutte le variabili (specie) presenti neanche a seguito della trasformazione logaritmica, si è deciso di ricorrere a metodi non parametrici in cui le analisi avvengono sui ranghi (Soliani, 2005).

**Tab. 1:** Giornate di attività didattica svolte all'interno della Riserva Marina di Miramare e date dei campionamenti effettuati nel periodo corrispondente.

**Tab. 1:** Datumi dejavnosti, povezanimi z okoljskim izobraževanjem v Morskem rezervatu Miramare, in datumi aktivnosti, povezanimi z monitoringom.

Giornate con attività didattiche	Date dei campionamenti nei periodi
	02/08/2004
	20/08/2004
	18/09/2004
	29/09/2004
13/10/2004	13-17/10/2004
09-12/11/2004	11/11/2004
	13/12/2004
	24/12/2004
	10/01/2005
24-25/01/2005	
	08-09/02/2005
07-08/03/2005, 10-11/03/2005	10/03/2005
19/03/2005, 21-22/03/2005	23/03/2005
02/04/2005, 05-07/04/2005	05/04/2005
04-06/05/2005	
19-20/05/2005	26/05/2005
	11/07/2005
	20/09/2005
06/10/2005	
14/10/2005, 17-20/10/2005	
	02/11/2005
04/11/2005	
	16/11/2005
28/11/2005	
	01/12/2005
13-15/12/2005	14/12/2005
	30/12/2005
	11/01/2006
23/01/2006	
06/02/2006, 10/02/2006, 14-15/02/2006	
23/02/2006	
02/03/2006	
13-15/03/2006, 17/03/2006	13/03/2006
23-24/03/2006, 27-28/03/2006	
07-08/04/2006, 10-12/04/2006	
20-21/04/2006, 24/04/2006	28/04/2006
19/05/2006	17/05/2006

Il test ANOSIM (ANalysis Of SIMilarities; Clarke, 1993) utilizzato per valutare la differenza significativa tra i due gruppi basata su misure di distanza di Bray-Curtis convertite in ranghi, verifica l'ipotesi nulla che la media della dissimilarità dei ranghi tra le possibili coppie di oggetti in gruppi differenti sia uguale alla media della dissimilarità dei ranghi tra le coppie di oggetti all'interno dello stesso gruppo. Il valore R che se ne ricava è compreso tra -1 e +1 con valori positivi indicanti che la dissimilarità tra i gruppi è maggiore di quella all'interno del gruppo (Quinn & Keough, 2002). Nello specifico dal calcolo effettuato sulla tabella delle abbondanze medie rinvenute si osserva una differenza statisticamente significativa tra il gruppo di stazioni sottoposte al calpestio e quello delle stazioni chiuse alla fruizione con un valore di  $R = 0,22$  e una probabilità di uguaglianza  $p < 0,0001$ .

Allo scopo di ottenere un ordinamento su un piano bidimensionale delle stazioni di campionamento in base ai popolamenti rinvenuti, la tabella delle abbondanze medie per i 35 taxa censiti è stata quindi sottoposta ad un'analisi di tipo nMDS (non-metric Multi Dimensional Scaling; Kruskal & Wish, 1978), tecnica molto utilizzata nei lavori di valutazione di influenze antropiche sui popolamenti costieri (Gambi & Dappiano, 2003). Clarke & Warwick (2001), inoltre, suggeriscono come un ordinamento nMDS sia in grado di evidenziare meglio della classificazione gerarchica (cluster analysis) la posizione di grandi gruppi gli uni rispetto agli altri.

L'analisi si basa sul calcolo di una matrice di distanze ottenute secondo l'equazione di Bray-Curtis (Bray & Curtis, 1957). Il grafico che se ne ricava riporta i punti caratterizzanti le stazioni di campionamento in uno spazio bidimensionale in modo che la distanza tra i ranghi ottenuti sia mantenuta.

## RISULTATI

Dai campionamenti effettuati si sono identificati 35 taxa diversi alcuni riconosciuti a livello di specie ed altri invece a livello sistematico superiore. Il riconoscimento infatti è avvenuto a vista direttamente sul sito di campionamento e quindi per alcuni organismi l'identificazione specifica è risultata impossibile. Trattandosi, infatti, di una ricerca svolta all'interno di un'area marina protetta si è dovuto rispettare il criterio di non invasività della metodologia di campionamento e raccolta dati. La distribuzione tassonomica è così rappresentata: 11 molluschi (poliplacofori, gasteropodi e bivalvi), 9 crostacei (anfipodi e decapodi), 4 policheti (sedentari ed erranti), 3 celenterati, 2 pesci, 2 poriferi, 1 echinoderma, 1 ascidiaceo, 1 briozoo e 1 platelminto.

### L'analisi statistica sulle specie campione

I dati organizzati in una tabella generale delle ab-

bondanze rinvenute nei 2 siti di indagine durante il periodo che va da agosto 2004 a maggio 2006, individuano la presenza di pochi taxa numericamente dominanti. Per uniformare il peso di questi taxa nell'elaborazione successiva, i dati sono stati trasformati secondo la funzione logaritmica  $y = \ln(x + 1)$ .

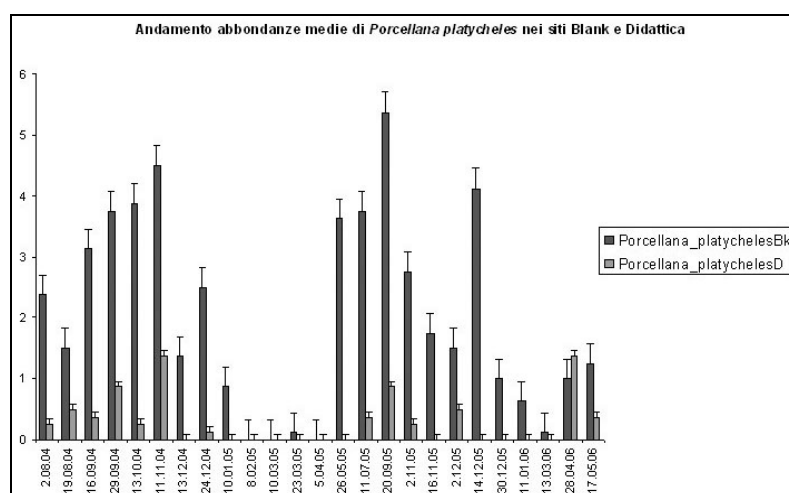
Un'analisi della varianza (ANalysis Of VAriance) è stata effettuata per verificare l'ipotesi nulla che non ci siano differenze nella media della distribuzione degli organismi tra i due siti. Per discriminare tra l'area calpestata e quella non calpestata si sono presi in esame come organismi rappresentativi solo i 10 taxa che hanno dimostrato avere una distribuzione normale delle abbondanze, trasformate secondo la funzione logaritmica, nell'arco del periodo di campionamento.

I taxa indagati sono stati *Porcellana platycheles*, *Chthamalus* spp., *Balanus* sp., Amphipoda indet., *Mytilus galloprovincialis*, *Monodonta turbinata*, *Patella caerulea*, *Serpula vermicularis*, *Spirorbis* sp., *Mytilaster minimus* e di questi i primi quattro hanno dimostrato una distribuzione significativamente differente tra il sito Didattica e il sito Blank (Tab. 2). Si tratta quindi di due taxa sessili legati alla presenza di grossi ciottoli nell'area campionata (*Chthamalus* spp., *Balanus* sp. – i comuni denti di cane) e di due taxa mobili che normalmente vivono tra gli interstizi lontano dalla luce e che se vengono portati allo scoperto tendono a spostarsi velocemente in punti rimasti ancora riparati (*P. platycheles* – il granchio dalle chele piatte, Amphipoda indet. – le pulci di mare).

**Tab. 2: Analisi della varianza sull'abbondanza di 8 taxa nei due siti: Didattica e Blank.**

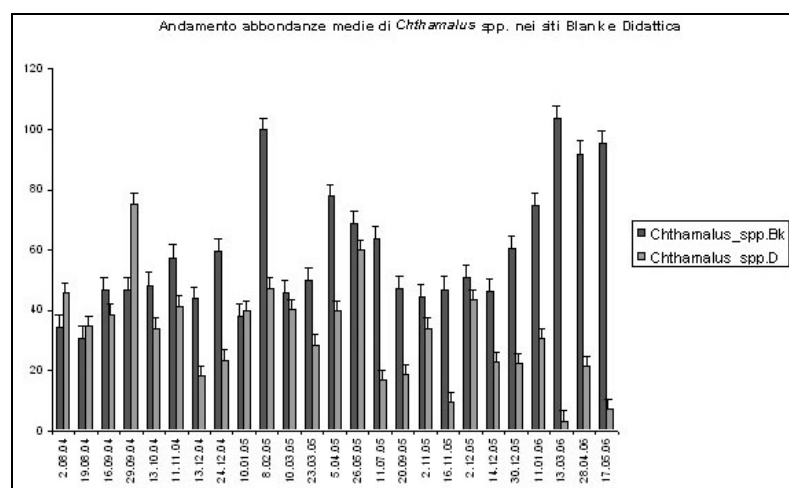
**Tab. 2: Analiza sprememb v številčnosti 8 taksonov na dveh vzorčičih: Didattica (vzorčiče za didaktične namene) in Blank (kontrolno vzorčiče).**

Origine di varianza	df = 1	Didattica vs. Blank		
		MS	F	p
<i>Porcellana platycheles</i>	***	1,27	31,43	<0,001
<i>Chthamalus</i> spp.	***	1,16	23,87	<0,001
Amphipoda indet.	**	0,974	7,5	<0,01
<i>Balanus</i> sp.	*	0,15	4,99	<0,05
<i>Mytilus galloprovincialis</i>		0,226	2,22	0,143
<i>Monodonta turbinata</i>		0,125	0,75	0,39
<i>Patella caerulea</i>		0,01	0,1	0,75
<i>Serpula vermicularis</i>		0,0004	0,001	0,97
<i>Spirorbis</i> sp.		0,008	0,13	0,72
<i>Mytilaster minimus</i>		0,17	2,04	0,16



**Fig. 5:** Andamento abbondanze medie di *Porcellana platycheles* nei siti Blank e Didattica durante il periodo di campionamento che va da agosto 2004 a maggio 2006.

**Sl. 5:** Trend povprečne številčnosti vrste *Porcellana platycheles* na kontrolnem vzorčiču (Blank) in vzorčiču za didaktične namene (Didattica) v obdobju med avgustom 2004 in majem 2006.



**Fig. 6:** Andamento abbondanze medie di *Chthamalus* spp. nei siti Blank e Didattica durante il periodo di campionamento che va da agosto 2004 a maggio 2006.

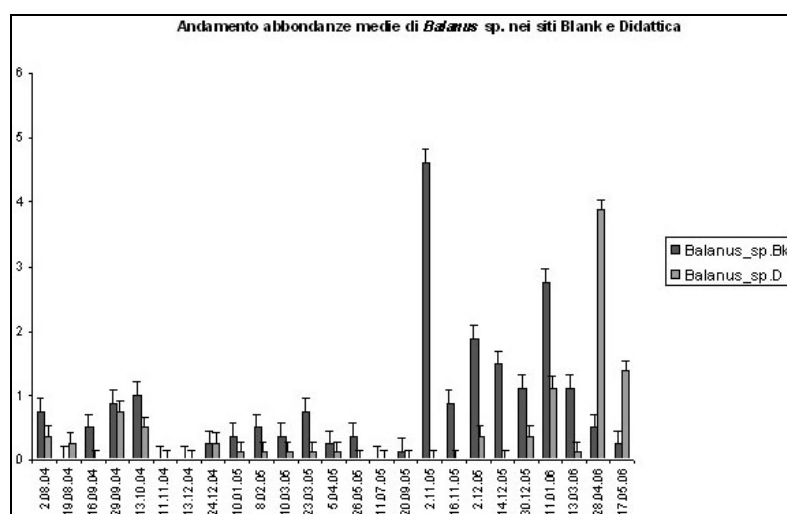
**Sl. 6:** Trend povprečne številčnosti *Chthamalus* spp. na lokalitetah Blank in Didattica v vzorčičnem obdobju med avgustom 2004 in majem 2006.

*P. caerulea* e *M. turbinata* mostrano un andamento di abbondanze simile nei due siti e sembrano quindi non essere soggette al disturbo, confermando le osservazioni raccolte in studi analoghi per specie affini (Jenkins et al., 2002).

Analizzando i grafici della distribuzione delle abbondanze medie nei due siti per le quattro specie che hanno dimostrato una differenza significativa secondo l'analisi della varianza, si nota come la loro abbondanza sia sempre maggiore nel sito chiuso alla fruizione rispetto a quello in cui c'è attività didattica e quindi calpestio per

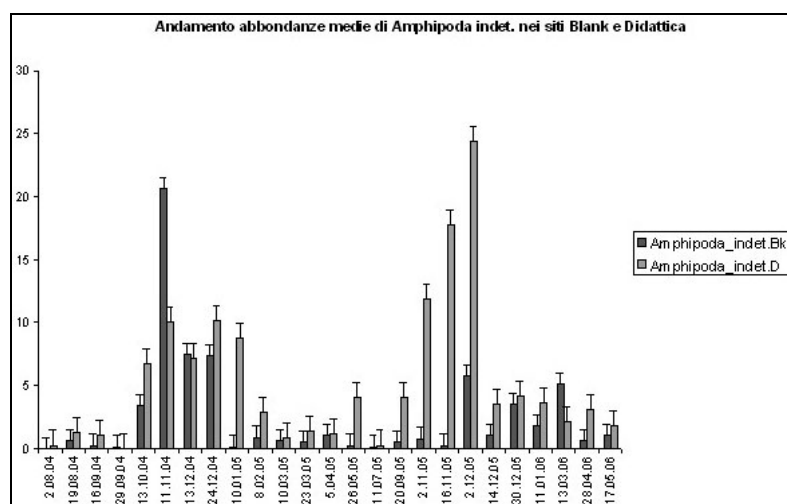
le specie *P. platycheles* (Fig. 5), *Chthamalus* spp. (Fig. 6), *Balanus* sp. (Fig. 7).

Amphipoda indet. (Fig. 8) invece mostra un andamento contrario e cioè è presente quasi in tutti i campionamenti in quantità media superiore nel sito Didattica rispetto al sito Blank. Inoltre, gli anfipodi hanno abbondanze maggiori durante il periodo autunnale ed invernale probabilmente in coincidenza con la minor presenza di fruitori. Studi specifici su una specie di anfipode (*Talitrus saltator*) in due aree, una protetta e l'altra con presenza di turisti, hanno indicato che ad una



**Fig. 7:** Andamento abbondanze medie di *Balanus* sp. nei siti Blank e Didattica durante il periodo di campionamento che va da agosto 2004 a maggio 2006.

**Sl. 7:** Trend povprečne številčnosti *Balanus* sp. na lokalitetah Blank in Didattica v vzorčičnem obdobju med avgustom 2004 in majem 2006.



**Fig. 8:** Andamento abbondanze medie di *Amphipoda* indet. nei siti Blank e Didattica durante il periodo di campionamento che va da agosto 2004 a maggio 2006.

**Sl. 8:** Trend povprečne številčnosti nedoločenih amfipodnih rakov (*Amphipoda* indet.) na lokalitetah Blank in Didattica v vzorčičnem obdobju med avgustom 2004 in majem 2006.

loro scomparsa durante il picco di presenza turistica segue una ricolonizzazione con la diminuzione e/o assenza del disturbo antropico (Fanini, 2005).

I punti sul grafico nMDS (Fig. 9), ottenuto dall'analisi multivariata, individuano le stazioni Blank con un quadrato vuoto e le stazioni Didattica con una croce. Osservando le stazioni del gruppo Blank e quelle del gruppo Didattica nel loro insieme in Fig. 9 si nota come tutti i punti relativi alle stazioni non calpestate (Blank) si posizionino a sinistra della linea immaginaria di divisione dello 0 sull'asse della coordinata 1 ad eccezione

dei campionamenti del 19/08/2005, del 13/12/2004 e 10/01/2005 che si trovano sulla destra. Conseguentemente tutti i punti relativi alle stazioni soggette a calpestio (Didattica) si trovano nello spazio a destra della linea dello 0 sulla coordinata 1 ad eccezione di sei campionamenti (D02/08/2004, D29/09/2004, D09/02/2005, D26/05/2005, D10/03/2005, D11/07/2005).

Occorre sottolineare che il livello di stress sul Shepard plot è di 0,15, valore che suggerisce una rappresentazione grafica con una buona capacità descrittiva.



recupero e di ripopolamento grazie alla presenza di individui giovanili. Va sottolineato che le attività di visita si svolgono soprattutto tra marzo e maggio in un momento quindi in cui la capacità del sistema di riprendersi è migliore.

Considerando poi che le attività svolte dai ragazzi possono essere paragonate ad attività di campionamento su aree di 1 m<sup>2</sup> scelte a caso nella zona della spiaggia delle ex-Scuderie, si può ritenere, come indicato anche da studi bibliografici riguardanti l'incidenza del disturbo sulla comunità dovuto all'azione di campionamento, che esso sia limitato al breve termine e che dopo poche settimane la comunità si ricostituisca (Chapman & Underwood, 1996). Anche analisi svolte valutando la ricchezza specifica di siti all'interno di aree protette e di siti accessibili al pubblico per scopi ricreativi (Jenkins *et al.*, 2002), indicano come la ricchezza specifica nella zona disturbata ritorni ai livelli di quella della zona protetta dopo un mese dalla fine del disturbo.

In ogni caso lo studio ha reso possibile anche una caratterizzazione quali-quantitativa della comunità

inframareale, nello specifico della Riserva marina di Miramare, ambiente che rimane ancora poco studiato a livello mediterraneo probabilmente perché a questa scala non risulta essere particolarmente rappresentativo. Le fonti bibliografiche rinvenute sono infatti piuttosto scarse e più facilmente legate allo studio di singole specie o comunità (Benedetti-Cecchi *et al.*, 1996, 2003; Menconi *et al.*, 1999) piuttosto che indagare sugli impatti antropici.

## RINGRAZIAMENTI

E' doveroso ringraziare tutti i componenti dello staff della Riserva Naturale Marina di Miramare per aver messo a disposizione il personale, i mezzi e le strutture per lo svolgimento del presente lavoro. Un sentito grazie a tutti coloro che si sono avvicendati nell'aiuto e supporto alla fase di raccolta dati che in due anni di campionamenti è risultata impegnativa: in particolare la dott.ssa Morgana Tagliarolo, la dott.ssa Anna Sustersic, la dott.ssa Gianna Visintin, la dott.ssa Alessia Malusà.

## PRELIMINARNA OCENA POSLEDIC POTEPTANOSTI OBREŽJA V MORSKEM REZERVATU MIRAMARE: MONITORING BIBAVIČNEGA PASU

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## POVZETEK

Med avgustom 2004 in majem 2005 so avtorji opravljali monitoring bibavičnega pasu v Morskem rezervatu Miramare. Njihov namen je bil oceniti morebitne vplive poteptanosti obrežja s strani šolskih skupin med izobraževalnimi dejavnostmi na organizme v bibavičnem pasu. Izbrani sta bili dve vzorčiščni območji na peščenem in prodnatem obrežju znotraj zaščitenih cone: prva (imenovana Blank) je bila eksperimentalno zaščiten pred poteptanostjo, druga (imenovana Didattica) pa prepuščena teptanju s strani šolskih skupin med njihovimi izobraževalnimi dejavnostmi.

Zbiranje podatkov je potekalo med oseko (ko je bilo vodno površje najmanj 40 cm pod srednjim nivojem morja) na skupaj 25 vzorčiščih v obeh območjih, in sicer z uporabo metode vzorčenja z vizualno identifikacijo in določitvijo vrst *in situ* ter tudi štetjem posameznih osebkov. Metodologija je morala biti uporabljena na neinvaziven način, saj je potekalo v zaščitenem morskem območju.

Analiza testa varianc (ANOVA) pri organizmih, prešteti na obeh vzorčiščnih območjih, je pokazala razlike v številčnosti pri 4 taksonih: *Porcellana platycheles*, *Chthamalus spp.*, *Balanus sp.*, *Amphipoda* indet. Taksoni *P. platycheles*, *Chthamalus spp.* in *Balanus sp.* so bili zabeleženi predvsem v območju, zaprtem za javnost (Blank), medtem ko je bil *Amphipoda* indet. številčnejši na poteptani lokaciji (Didattica).

Multivariatna analiza nMDS (nemetrično multidimenzionalno skaliranje), ki razporeja točke v dvodimenzionalni prostor na podlagi matrike oddaljenosti, izračunane s podatki o skupni abundanci, je pokazala razliko med lokalitama Blank in Didattica, ki sta nagnjeni k formiranju dveh različnih skupin, čeprav nekatere postaje dveh grupacij, predvsem tistih v poteptanem območju, ležijo znotraj nasprotne skupine, kar kaže na večjo podobnost s slednjo. Na splošno je bila ugotovljena večja konstantnost populacije v nepoteptanem območju, medtem ko je bila večja variabilnost zabeležena v poteptanem območju med obdobjem vzorčenja.

*Dobljeni rezultati namigujejo, da je morebitni nemir znotraj združbe v bibavičnem pasu trenutno omejen v prostoru in kratkotrajen zaradi teptanja med izobraževalnimi dejavnostmi.*

**Ključne besede:** zaščiteno morsko območje, združba bibavičnega pasu, antropogeni vpliv, teptanje, nMDS, monitoring

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## CHARACTERIZATION OF THE NOISE PRODUCED BY CLASS 1 POWERBOAT RACE IN PIRAN BAY (SLOVENIA) AND POTENTIAL IMPACT ON THE MARINE FAUNA

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### ABSTRACT

*Underwater noise produced during a Class 1 powerboat race was measured in the Bay of Piran (Slovenia) in September 2006. The calculated  $L_{Leq\ 30\ sec}$  of a single powerboat passing 300 m away from the hydrophone was equal to 120 dB re 1  $\mu$ Pa, exceeding the local sea ambient noise by an average of 8.5 dB within the considered frequency range (40–22100 Hz). The same powerboat, passing 670 m away, allowed the estimation of the source level, which is 145 dB re 1  $\mu$ Pa at 1 m. Comparisons with the hearing abilities of a crustacean, a fish species, a marine mammal, and indications from previous studies lead us to conclude that noise of such intensity can elicit behavioural responses as well as mask and impair acoustic communication in the considered animals. We also noticed that some 200–300 spectator's boats moving across the race field simultaneously at the end of the race brought an increment in the sea ambient noise exceeding the powerboat noise in the frequency range below 500 Hz.*

**Key words:** noise pollution, anthropogenic impact, power boat race, Adriatic Sea

## CARATTERIZZAZIONE DEL RUMORE IRRADIATO IN ACQUA DA MOTOSCAFI TIPO OFFSHORE CLASSE 1 IN COMPETIZIONE NELLA BAIA DI PIRANO (SLOVENIA) E POTENZIALI IMPATTI SULLA FAUNA MARINA

### SINTESI

*Il rumore subacqueo prodotto da motoscafi tipo offshore Classe 1 è stato misurato nel corso di un Gran Premio del campionato mondiale, tenutosi nel settembre 2006 nella baia di Pirano (Slovenia). L' $L_{Leq\ 30\ sec}$  calcolato per un singolo motoscafo che transitava a 300 m di distanza dall'idrofono era uguale a 120 dB re 1  $\mu$ Pa, superando mediamente il sea ambient noise locale di 8,5 dB nel range di frequenze considerato (40–22100 Hz). Tenuto conto del livello di pressione sonora generato dalla stessa imbarcazione che passava ad una distanza di 670 m, si è potuto stimare il source level, che è di 145 dB re 1  $\mu$ Pa a 1 m. La comparazione della sensibilità acustica di diverse specie marine – un crostaceo, un pesce ed un mammifero – assieme ad indicazioni tratte da studi precedenti, permette di concludere che rumori di tale intensità possono determinare variazioni di tipo comportamentale, nonché mascherare e rendere difficoltosa la comunicazione acustica negli animali considerati. Inoltre, è stato rilevato che circa 200–300 barche con a bordo spettatori, le quali si muovevano simultaneamente lungo il confine del campo di gara alla fine della competizione, portano ad un incremento del rumore ambiente subacqueo locale, superando il rumore prodotto da un motoscafo nel range di frequenze al di sotto dei 500 Hz.*

**Parole chiave:** inquinamento acustico, impatto antropico, gara di motoscafi tipo offshore, mare Adriatico

## INTRODUCTION

A constant increase of the human activities in many diverse natural environments has pointed out the necessity of monitoring and evaluating the potential effects that human presence may cause. Among others, noise pollution is a serious threat to marine animals whose effects, compared to more visible pollutants like oil spills and marine debris, are not as easy to notice. Even if they are noticed, they cannot be easily stopped and confined out of sensitive or protected areas. Noise pollution is particularly relevant in coastal areas due to the high number of anthropogenic sources as, for example, the motorised vessels (Allen & Read, 2000; Buckstaff, 2004). Several studies showed that boat noise elicit different types of avoidance behavior both in small cetaceans and in fish (e.g., Avecedo, 1991; Nestler *et al.*, 1992; Janik & Thompson, 1996; Gregory & Rowden, 2001; Mitson & Knudsen, 2003; Sarà *et al.*, 2007). Temporary hearing loss (Scholik & Yan, 2001; Amoser & Ladich, 2003; Smith *et al.*, 2004), impaired temporal resolution ability (Wysocki & Ladich, 2005), damages to the sensory epithelia of the inner ear (Hastings *et al.*, 1996; McCauley *et al.*, 2003) and endocrinological stress responses (Santulli *et al.*, 1999; Smith *et al.*, 2004) caused by exposure to different anthropogenic noise types have been also demonstrated in these taxa.

A Class 1 Powerboat Race, part of the world offshore championship, took place out of Piran Bay (Slovenia) between 1<sup>st</sup> and 3<sup>rd</sup> September 2006. About 10 offshore powerboats participated in the competition; these are boats with a high power to weight ratio and a hull with twin inboard engines designed for easy planning, high speed (up to 270 km/h; Amoser *et al.* 2004) and improved handling. Although the offshore competitions are raced every year on three different continents, only few environmental impact assessments have been made on them. Despite being limited in number, these assessments pointed out a particular concern about hydrological forces and turbidity in view of the blue mussels, about potential collisions between powerboats and marine mammals and about fish disturbance due to the high noise levels emanating from the vessels (Morgenroth, 2002, 2003; Amoser *et al.*, 2004).

The aims of the present study are (1) to describe underwater noise emissions and noise levels produced by the powerboats and (2) to compare them with the local sea ambient noise recorded both in quiet and boat traffic condition. The hearing abilities of a crustacean, a fish species and a marine mammal, whose home ranges include the competition area, are taken into account in order to discuss the potential impacts of the powerboat noise.

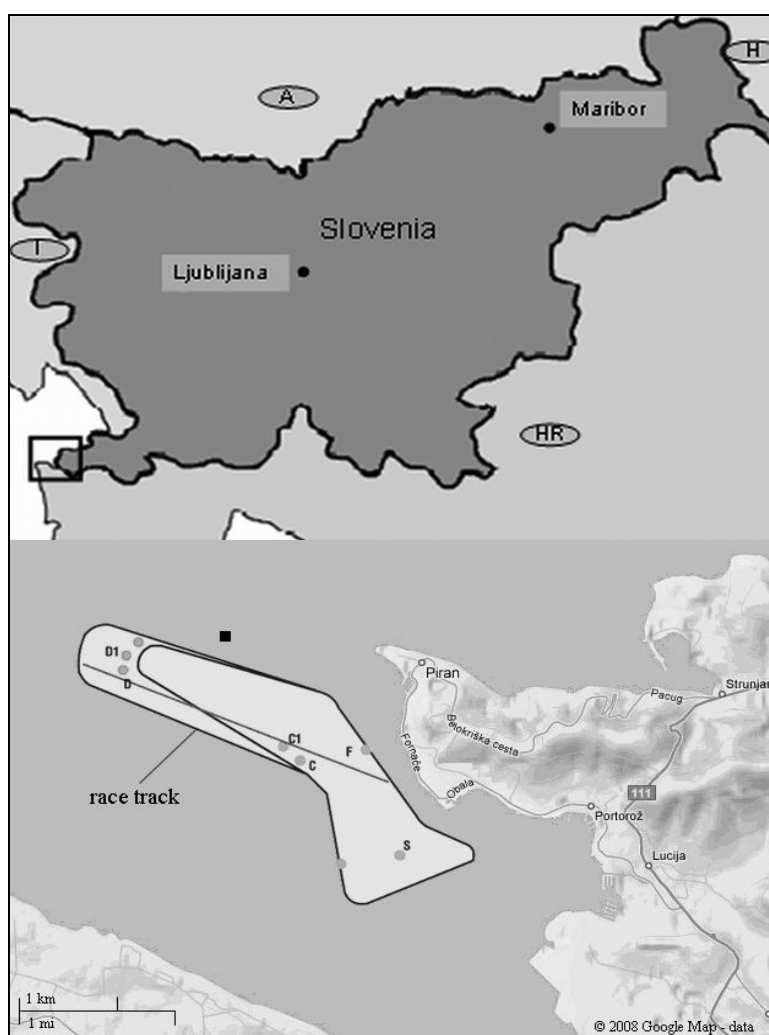
## MATERIALS AND METHODS

The race was run in Slovenian waters of the Trieste

Gulf (North Adriatic Sea), 2 km outside the town of Piran. Data were collected from a rubber boat from 10 to 11.30 a.m. on 2 September 2006 (pole position) and from 14 to 16 p.m. on 3 September 2006 (race). The rubber boat was anchored during both days at a distance of approximately 300 meters from the longest straight leg of the race (Fig. 1; 45°31'48.54" N, 13°33'14.84" E), where powerboats passed at highest speed. This was the place closest to the race, due to an official security zone. The weather was sunny on both days (sea state 0), with water temperature ranging from 23°C (surface) to 20°C (at 15 meters depth).

The noise emission of the powerboats was recorded at a depth of 10 m (muddy bottom; depth 20 m) using a calibrated Reson TC4032 hydrophone (Slangerup, Denmark; sensitivity –170 dB re 1 V/μPa) and a Pioneer D-C88 DAT recorder (Pioneer Electronics, U.S.A.; sample rate 44.1 kHz, 16-bit) battery operated. These settings were used also for recording the sea ambient noise before the pole, when no powerboats were running and a limited number of motor boats (< 10) were visible in Piran Bay (quiet condition, QC), and after the race, when about 200–300 boats of public viewers were moving along the race circuit (traffic condition, TC).

All the recordings were analysed in terms of instantaneous sound pressure levels (SPL, *L*-weighted, 20 Hz to 20 kHz, RMS fast) using Spectra RTA (Sound Technology) spectral analyser calibrated with a signal of 100 mV rms @1 kHz and hydrophone sensitivity. For the analysis, ten 30 sec-samples were chosen from the race noise recordings; all of them include the background noise recorded while single powerboats were passing at the minimum distance ( $300 \pm 10$  m; powerboat close, PC) or at the maximum distance ( $670 \pm 10$  m; powerboat far, PF) from the hydrophone. Distances were measured through a hand-hold GPS navigator, by crossing the race ground right after the competition, while the buoys setting the lanes were still in place. Their  $L_{Leq}$  values (30 sec) were calculated as well as their power spectra and the loudest and weakest samples (corresponding to the minimum and maximum distance of the powerboat) were considered for comparative analysis with the sea ambient noise. 30 sec-samples of ambient noise were also randomly chosen both for quiet and traffic condition and their  $L_{Leq}$  (30 sec) calculated. The loudest and weakest samples were considered for comparative analysis: the instantaneous sound pressure level ( $L_{LSP}$ , *L*-weighted, 10 Hz to 20 kHz, RMS fast) was calculated over a 30 sec sample ( $n=30$  for each sample) per each of the four conditions (PF, PC, QC, TC) and a multivariate ANOVA based on two within-subjects factors (frequency and condition) with a Bonferroni post-hoc test was applied on the data using the computer package Statistica 6.0 for Windows (StatSoft, Inc.). Assumptions of normality and homogeneity of variances were met. The sonogram and the waveform of the powerboat noise



**Fig. 1: Race track of the Class 1 powerboat race in Piran Bay. The black square indicates the recording position.**

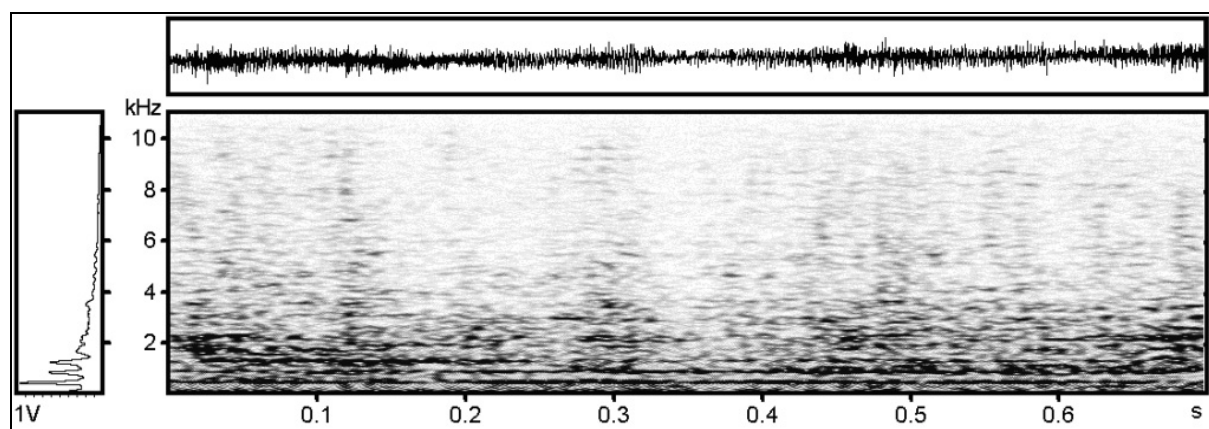
**Sl. 1: Tekmovalna proga za motorne čolne razreda 1 v Piranskem zalivu. Črni kvadrat označuje mesto opravljenih meritev.**

were displayed using Avisoft SasLab Pro (Avisoft Bioacoustics, Berlin, Germany) signal processing software (Hamming window, frequency bandwidth of 75 Hz, 1024 point FFT, overlap 97%, frame 75%).

## RESULTS

One powerboat passing about 300 m from the hydrophone (PC) produces a noise that is concentrated mainly below 5 kHz with a maximum instantaneous sound pressure level (SPL) of 126 dB re 1  $\mu$ Pa. From the sonogram and the power spectra of the noise (Fig. 2) three harmonics are easily detectable. The mean fundamental frequency is equal to  $420 \pm 4.7$  Hz (range 412–426 Hz,  $n=10$ ), corresponding to the rotational speed of the propeller.

Figure 3 represents the power spectra of the four considered conditions (PF, PC, QC, TC). The harmonic peaks are evident in the spectra of the powerboat noise recorded at 300 m (PC) and at 670 m (PF) of distance. PC spectra levels remain much higher in the upper frequency range than all the other conditions. This is not the case of PF spectra that show a decrement at high frequency similar to QC and TC. This likely occurred because the noise transmission loss is frequency dependent, and attenuation of sound by seawater increases with increasing frequency (Rogers & Cox, 1988). On the opposite, the main energy in the low frequency range (<400 Hz) is produced by the boat traffic present in the area after the race (TC). As predictable, QC has the quietest spectra. Compared to quiet conditions (QC), PC noise shows an almost constant increment of  $8.5 \pm 3.7$  dB along all the frequencies ( $n=10$ ).

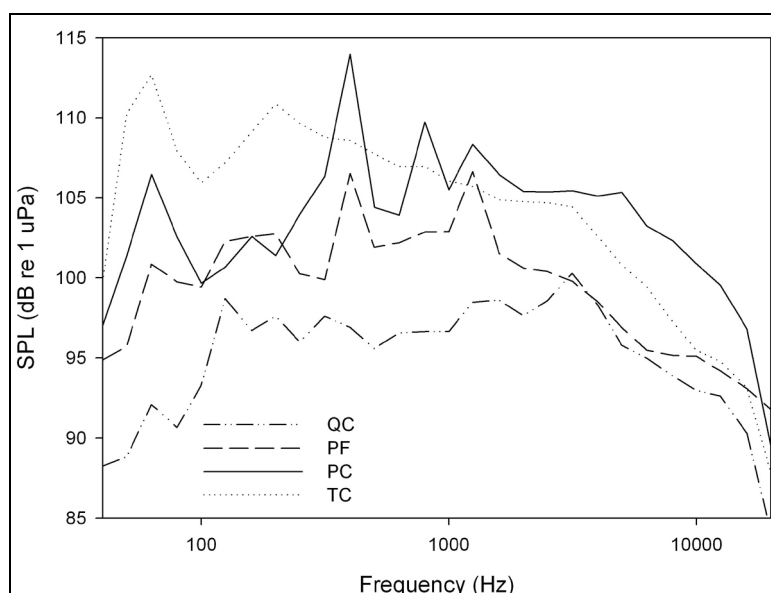


**Fig. 2:** Sonogram, power spectra (on the left) and oscillogram (above) of the noise produced by a powerboat passing at 300 m from the hydrophone (Hamming window, frequency bandwidth of 75 Hz, 1024 point FFT, overlap 97%, frame 75%).

**Sl. 2:** Sonogram, spektri moči (na levi) in oscillogram (zgoraj) hrupa, ki ga je povzročil motorni čoln na razdalji 300 m od hidroфона (Hammingovo okno, širina frekvenčnega pasu 75 Hz, 1024 točk FFT, prekrivanje 97%, okvir 75%).

The calculated  $L_{Leq}$  (30 sec) for each of the four considered conditions (PF, PC, QC, TC) is equal to 120 dB re 1  $\mu$ Pa (PC), 116 dB re 1  $\mu$ Pa (PF), 111.3 dB re 1  $\mu$ Pa (QC) and 119.8 dB re 1  $\mu$ Pa (TC). Although some values can be considered similar, a multivariate ANOVA re-

vealed a significant effect of the four conditions (PF, PC, QC, TC) on their  $L_{LSP}$  calculated over 30 sec ( $F=1766$ ;  $P<0.001$ ) and the post-hoc Bonferroni test indicates that each condition differs significantly from all the others ( $P<0.001$ ).



**Fig. 3:** Power spectra of the different recorded noise. QC = sea ambient noise in quiet condition; TC = sea ambient noise in traffic condition; PC = noise produced by a powerboat passing at a distance of 300 m from the recording point; PF = noise produced by a powerboat passing at 670 m distance from the recording point. See text for details.  
**Sl. 3:** Spektri moči različnega hrupa. QC = hrup lokalnega morskega okolja v mirnih razmerah; TC = hrup lokalnega morskega okolja v razmerah povečanega morskega prometa; PC = hrup, ki ga je povzročil motorni čoln na razdalji 300 m od točke merjenja; PF = hrup, ki ga je povzročil motorni čoln na razdalji 670 m od točke merjenja. Podrobnosti v besedilu samem.

## DISCUSSION AND CONCLUSIONS

Boat and ships are major contributors to the overall man-made noise in the sea, given their large numbers, wide distribution and mobility. Sea traffic noise characterizes the sea ambient noise of coastal areas mainly in the range below 1 kHz, nevertheless boat noise source level (*i.e.* the amount of radiated sound at a particular frequency measured at 1 m from the source) and frequency characteristics are extremely variable in relation to speed, load, pitch angle of propeller and age of the vessel (Mitson, 1993). Levels and frequencies of both tonal and broadband sounds tend to be related to vessel size, but are also strongly affected by vessel design and speed (Richardson *et al.*, 1995). In general, source levels of small boats increased with increasing speed (Erbe, 2002), whereas for large vessels (merchant cargo and passenger ships), the relationship is logarithmic (Ross, 1976). At high speed, propeller cavitation produces most of the broadband noise, with dominant tones arising from the propeller blade rate, whereas at low speeds, wave splashing and engine noise are usually audible as the prime component of the noise (Ross, 1976).

The present paper indicates a  $L_{\text{Leq}}$  (30 sec) of 120 dB re 1  $\mu\text{Pa}$  for a powerboat passing at 300 meter of distance and a maximum instantaneous SPL of 126 dB re 1  $\mu\text{Pa}$ , according to Amoser *et al.* (2004), that reported a maximum noise levels of 128 dB re 1  $\mu\text{Pa}$  (instantaneous SPL) generated by powerboats running at a distance of 300 m. This noise level is considerably louder than sea ambient noise recorded in the area in quiet condition (111.3 dB re 1  $\mu\text{Pa}$ ); on the other hand, it is similar to the background noise measured in concomitance of elevated boat traffic (119.8 dB re 1  $\mu\text{Pa}$ ). The noise produced by boat traffic is mainly concentrated below 500 Hz, whereas the powerboat noise shows harmonic peaks and a high energy component at high frequency. A significant difference between all the considered noise conditions in terms of instantaneous SPL is confirmed by the multivariate ANOVA. The post-hoc Bonferroni test demonstrates frequency-dependent variation of the background noise according to the number and types of vessels present in the area.

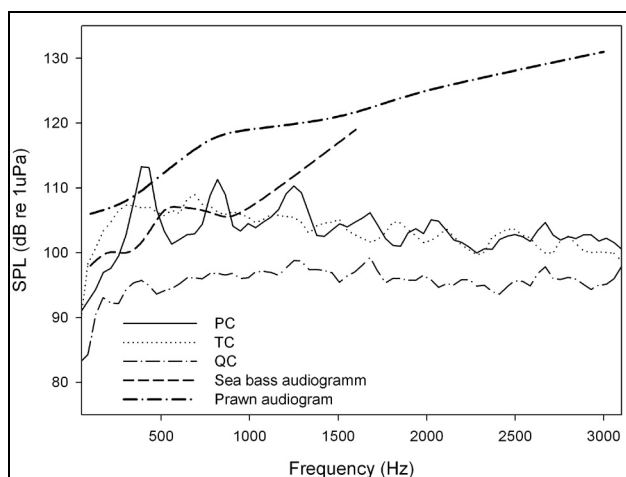
Amoser *et al.* (2004) indicate a source level (SL) of the powerboat equals to 180 dB re 1  $\mu\text{Pa}$  at 1 m distance, assuming a spherical noise spreading model ( $20 \log R$ , where  $R$  is the distance between noise source and hydrophone). On the other hand, we considered a cylindrical spreading ( $10 \log R$ ) as the best transmission loss model in shallow water (Richardson *et al.*, 1995), obtaining a source level of approximately 145 dB at 1 m. Although it must be considered an estimation, due to several other variables affecting the sound propagation in costal area (*i.e.* bottom morphology, absorption, shadow zones due to refraction, salinity, temperature clines, etc), this SL does not vary a lot from other re-

ported SL values for different ships. Following Boussard (1981), cruising barges and high speed boats determine 1/3 octave band levels of up to 140 and 160 dB, Vasconcelos *et al.* (2007) indicate a source level at 1 m of approx 143 dB being produced by a ferry boat, Erbe (2002) reported boat source levels ranging from 145 to 169 dB re 1  $\mu\text{Pa}$  at 1 m (an average of 162 dB re 1  $\mu\text{Pa}$  at 1 m for speeds of around 50 km/h), whereas Greene & Moore (1995) recorded a noise of 142 dB being produced by a 70 horsepower outboard motor at a distance of 50 m.

Moreover, we calculated that a SL of 145 dB at 1 m at a distance of 600 m generates a sound pressure level equal to 117 dB re 1  $\mu\text{Pa}$  according to the cylindrical model; this value fits well with the measured  $L_{\text{Leq}}$  (30 sec) of a powerboat passing at 670 m distance, confirming the validity of the model. This leads us to conclude that the powerboat noise is theoretically detectable above the local sea ambient noise (quiet condition, QC,  $L_{\text{Leq}}$  (30 sec) = 111.3 dB re 1  $\mu\text{Pa}$ ) for about 2 km from the source; up to this distance, we calculated a noise to ambient noise ratio larger than one.

Cylindrical spreading equation, however, does not account for variables such as source and receiver depth or complex bottom interactions; therefore we will consider only the noise values recorded in the field for discussing the potential impact of powerboat noise on the local fauna. Since the source level of the powerboat noise is surely higher than the here considered value, it is likely that the impacts are greater than those discussed here.

Figures 4 and 5 compare the sea ambient noise in the four conditions, *i.e.* sea ambient noise in quiet and traffic conditions (QC, TC) and noise produced by a powerboat when passing close and far from the hydrophone (about 300 and 670 m respectively; PC, PF), with the audiograms of a teleost fish, the sea bass (*Dicentrarchus labrax*), a crustacean, the prawn (*Palaemon serratus*) and a marine mammal, the bottlenose dolphin (*Tursiops truncatus*), which are present in the area of interest (Genov & Furlan, 2006). The sea bass is a hearing generalist fish (Lovell, 2003; Lovell *et al.*, 2005a) that lacks accessory hearing structures (air-filled cavities connected to the inner ear) and it is therefore mainly sensitive to particle motion components of low frequency sounds at relatively high sound intensities (Hawkins & Myrberg, 1983; Ladich & Popper, 2004). Its sound pressure audiogram reveals a maximum sensitivity of 98 dB re 1  $\mu\text{Pa}$  around 100 Hz and a hearing range up to about 1 kHz. The crustacean *P. serratus* has a perception of sound similar to hearing generalist fish, being responsive to signals ranging in frequency from 100 to 3000 Hz and having a peak sensitivity at about 100 Hz (Lovell *et al.*, 2005b). The comparison of the hearing sensitivities of these species with absolute powerboat spectra (Fig. 4) shows that they are not affected

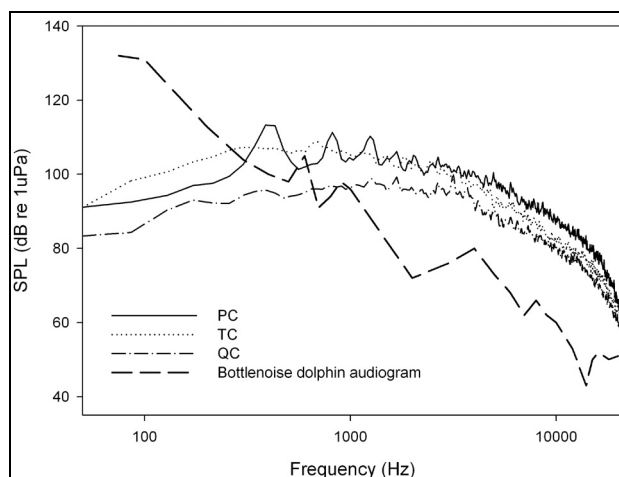


**Fig. 4: Audiograms of a hearing generalist fish, the sea bass (*Dicentrarchus labrax*; from Lovell, 2003), and a crustacean, the prawn (*Palaemon serratus*; from Lovell et al., 2005b), compared with different noise spectra. QC = sea ambient noise in quiet condition; TC = sea ambient noise in traffic condition; PC = noise produced by a powerboat passing at 300 m of distance from the recording point. See text for details.**

**Fig. 4: Avdiogrami morskih vrst – slušnih generalistov, brancina (*Dicentrarchus labrax*; po Lovellu, 2003) in žagaste kozice (*Palaemon serratus*; po Lovellu et al., 2005b), primerjani z različnimi spektri hrupa. QC = hrup lokalnega morskega okolja v mirnih razmerah; TC = hrup lokalnega morskega okolja v razmerah povečanega morskega prometa; PC = hrup, ki ga je povzročil motorni čoln na razdalji 300 m od točke merjenja. Podrobnosti v besedilu samem.**

by the QC. On the opposite, the increase in prevailing sea noise due to a powerboat passing at 300 m distance (PC) results in the signal detection being impaired. If masking is assumed to occur when the noise band levels are equal to or higher than the band levels of a signal, following Fletcher's equal-power-assumption (Fletcher, 1940), the sea bass and the prawn are likely to be masked mainly in the frequencies concomitant with the harmonics, which contain the peak energy of the noise generated by powerboat. The same effect has been demonstrated in other generalist fish by Amoser et al. (2004).

At low frequency range (below 500 Hz), however, the boat traffic seems to have an impact on the fish species, due to an increment of the ambient noise up to a maximum of about 8 dB above their hearing thresholds. A masking effect can be hypothesized in the best hearing range of the sea bass; boat noise negative impacts on hearing and acoustic communication have been already demonstrated in the Lusitanian toadfish (*Halobatrachus*



**Fig. 5: Audiograms of the bottlenose dolphin (*Tursiops truncatus*; from Johnson, 1968) compared with different noise spectra (x-axes in logarithmic scale). QC = sea ambient noise in quiet condition; TC = sea ambient noise in traffic condition; PC = noise produced by a powerboat passing at 300 m of distance from the recording point. See text for details.**

**Sl. 5: Avdiogrami velike pliskavke (*Tursiops truncatus*; po Johnsonu, 1968), primerjani z različnimi spektri hrupa (x-osi na logaritmčni lestvici). QC = hrup lokalnega morskega okolja v mirnih razmerah; TC = hrup lokalnega morskega okolja v razmerah povečanega morskega prometa; PC = hrup, ki ga je povzročil motorni čoln na razdalji 300 m od točke merjenja. Podrobnosti v besedilu samem.**

*didactylus*) (Vasconcelos et al., 2007), the brown meagre (*Sciaena umbra*) and the Mediterranean damselfish (*Chromis chromis*; Codarin et al., 2008).

The dolphin's audiogram (Johnson, 1967) showed a frequency range that extends from 75 Hz to 150 kHz with hearing sensitivity improving gradually with frequency up to a maximum sensitivity in the range between 15 kHz and 110 kHz, where acoustic communication takes place. According to figure 5, the bottlenose dolphin, *T. truncatus*, perceives all the here considered sea background noise conditions (QC, TC, PC, PF). Erbe & Farmer (1998) indicated that a propeller cavitation noise may completely mask a typical beluga (*Delphinapterus leucas*) localization for noise-to-signal ratios greater than 18 dB. Although it appears likely that the noise produced by a powerboat passing at 300 as well as 600 m distance can affect the bottlenose dolphin communication ability, a more accurate research should be done, exceeding the aim of the present paper. It must be considered, for example, that contrary to fishes, ma-

rine mammals are able to produce more calls, louder calls and shifting the frequency of their vocalization as a consequence of elevated background noise (Au, 1993; Foote *et al.*, 2004). From a behavioural point of view, a broadband sound pressure level of 120 dB re 1  $\mu$ Pa, as the one produced by a powerboat passing at a distance of 300 m, is used in marine mammals as a threshold of responsiveness (Richardson *et al.*, 1995). Following Erbe's models (Erbe, 2002), boat source levels that range from 145 to 169 dB re 1  $\mu$ Pa at 1 m are audible to killer whales over 16 km, mask killer whale calls over 14 km, elicit a behavioral response over 200 m, and cause a temporary threshold shift (TTS) in hearing of 5 dB after 30–50 min within 450 m. More in details, the playback of speedboats noise on bottlenose dolphins induced shorter surface periods, longer dives and movement away from vessels at ranges of 150–300 m (Evans *et al.*, 1992). Following Genov (2006), no apparent change in behaviour of bottlenose dolphin (either in terms of behavioural state, dive times, travel direction or sudden startle reaction) was observed during the training phase of the race in Piran Bay. Despite it, possible subtle reactions might not be visible at the surface and no data were available on the possible changes in the animals' acoustic behaviour (Genov, 2006). If we consider that the physical presence of boats impact the marine mammal behaviour (Blane & Jaakson, 1995; Janik & Thompson, 1996; Nowacek, 1999; Hastie *et al.*, 2003; Mattson *et al.*, 2005; Ribeiro *et al.*, 2005), a concern over the potential impacts of the powerboat race on this species can be expressed here.

The present study indicates that powerboats generated noise levels of a minimum of 145 dB re 1  $\mu$ Pa at 1 m distance during the first Class 1 powerboat race in Piran Bay, whose main energies are within the best hearing range of fish and crustacean species and are detectable by the bottlenose dolphin. Various authors in earlier studies indicate a behavioural response elicited by noise of such an intensity as well as masking and impaired acoustic communication. A cumulative effect of disturbance due to the simultaneous passages of many powerboats can also be hypothesized, whose implications are difficult to predict. We conclude that the noise produced by these vessels does disturb the local fauna. The extent of this disturbance needs a set of behavioural experiments to be assessed. At the end, it has to be noticed that some 200–300 spectator's boats moving simultaneously randomly across the race field determined an increment in the sea ambient noise exceeding the powerboat noise in the frequency range below 500 Hz. The impact of this noise should also be taken into account when running environmental impact assessments on powerboat races.

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## OZNAČITEV HRUPA, KI GA POVZROČAJO TEKMOVALNI ČOLNI RAZREDA 1 V PIRANSKEM ZALIVU, IN POTENCIALNI VPLIV HRUPA NA ŽIVLJENJE V MORJU

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#### POVZETEK

V začetku septembra 2006 so avtorji članka opravljali podvodne meritve hrupa, ki so ga povzročali čolni razreda 1 med motonavično dirko v Piranskem zalivu. Namen meritev je bil: 1) opisati podvodne emisije hrupa in ravni hrupa, ki ga povzročajo tekmovalni čolni, in 2) primerjati emisije hrupa s hrupom lokalnega morskega okolja, ki je

bil zabeležen tako v mirnih razmerah kot v razmerah s povečanim vodnim prometom. Hrup so merili z baterijsko napravo Pioneer D-C88 DAT in hidrofonom Reson TC4032, nameščenim v globini 10 m (pri tamkajšnji globini morja 20 m). Dan pred tekmo je bil izmerjen hrup lokalnega morskega okolja v mirnih razmerah, po koncu dirke pa v razmerah s povečanim vodnim prometom. Med dirko je bil izračunani  $L_{Leq, 30 \text{ sek}}$  enega čolna na razdalji 300 m od hidroфона enak 120 dB re 1  $\mu\text{Pa}$ , kar pomeni, da je presegel hrup lokalnega morskega okolja v mirnih razmerah s povprečno 8,5 dB znotraj frekvenčnega območja 40–22100 Hz. Hrup je skoncentriran predvsem pod 5000 Hz, srednja vrednost osnovne frekvence pa je  $420 \pm 4,7$  Hz, kar se ujema z vrtilno hitrostjo vijaka. Hrup istega tekmovalnega čolna na razdalji 670 m se je zmanjšal za 4 dB, s precejšnjim upadom v visokem frekvenčnem območju.

Izračunani nivo vira hrupa tekmovalnega čolna je torej enak 145 dB re 1  $\mu\text{Pa}$  pri 1 m. Pri tem lahko domnevamo, da je hrup tekmovalnega čolna v podobnih okoljskih razmerah teoretično mogoče zabeležiti nad hrupom lokalnega morskega okolja za približno 2 km od vira. To vrednost moramo tu upoštevati kot minimalno razdaljo, pri kateri je treba vir nemira obdržati proč od okoljsko občutljivih lokalitet (na primer morska zaščitena območja).

Primerjave s slušnimi sposobnostmi raka (žagasta kozice *Palaemon serratus*), ribe (brancina *Dicentrarchus labrax*) in morskega sesalca (velike pliskavke *Tursiops truncatus*), so hkrati z ugotovitvami iz prejšnjih študij vodile do sklepa, da hrup takšne jakosti lahko izsili določene vedenjske odzive pa tudi zastre in oslabi akustično komunikacijo pri treh izbranih živalih. Zaradi tako velikega nemira bi bilo treba nujno opraviti in oceniti niz vedenjskih eksperimentov.

Na koncu je bilo ugotovljeno, da je kakih 200 ali 300 čolnov zbranih gledalcev, ki so se takoj po dirki hkrati zapeljali prek tekmovalne proge, povzročilo hrup lokalnega morskega okolja, ki je v frekvenčnem območju pod 500 Hz presegal hrup, povzročen med dirko. In tudi ta hrup bi bilo treba upoštevati med ocenjevanjem vpliva motornavičnih dirk na okolje.

**Ključne besede:** zvočno onesnaževanje, antropogeni vpliv, dirka tekmovalnih čolnov, Jadransko morje

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## OPISTHOBRANCH MOLLUSCS (MOLLUSCA: GASTROPODA) FROM SLOVENIAN COASTAL WATERS (NORTHERN ADRIATIC)

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### ABSTRACT

*The present paper reports on the checklist of opisthobranch molluscs, found to inhabit Slovenian part of the Adriatic Sea. At least 66 opisthobranchs were recorded in the studied area and 74 in the entire Gulf of Trieste. To date, only a single non-indigenous species, Bursatella leachii, has been recorded in Slovenian coastal waters. The majority of species have been registered only in sporadic cases, and only few species have been found to be relatively common. Since only a small number of habitat types were investigated, the list of sea slugs of the Slovenian part of the Gulf of Trieste is to our opinion still far from complete.*

**Key words:** opisthobranch molluscs, checklist, Slovenia, Gulf of Trieste, Adriatic Sea

## MOLLUSCHI OPISTOBRANCHI (MOLLUSCA: GASTROPODA) IN ACQUE COSTIERE SLOVENE (ADRIATICO SETTENTRIONALE)

### SINTESI

*L'articolo riporta l'inventario dei molluschi opistobranchi ritrovati nella parte slovena del mare Adriatico. Nell'area studiata sono state identificate almeno 66 specie di opistobranchi, mentre 74 specie sono conosciute per l'intero Golfo di Trieste. Fino ad oggi una sola specie alloctona, Bursatella leachii, è stata segnalata in acque costiere slovene. La maggioranza delle specie è stata ritrovata solo in pochi casi, mentre poche specie sono risultate essere relativamente comuni. Durante lo studio solo un basso numero di habitat è stato perlustrato, pertanto gli autori sono convinti che la lista di molluschi opistobranchi del mare sloveno è tutt'altro che completa.*

**Parole chiave:** molluschi opistobranchi, inventario, Slovenia, Golfo di Trieste, mare Adriatico

## INTRODUCTION

To date, the opisthobranch fauna of the Slovenian coastal sea has been poorly investigated. Detailed data on the mollusc fauna of the Gulf of Trieste and the Slovenian part of this gulf were presented by Vio & de Min (1996) and De Min & Vio (1997), respectively. Unfortunately, some opisthobranch orders such as Notaspiidea, Anaspiidea and Nudibranchia were underestimated in terms of their numbers, since the authors have clearly used the basic malacological sampling techniques (such as Van Veen grabs, Petersen grabs), which are not appropriate for the study of sea slugs. To this end, only few representatives of the above orders were mentioned in their checklists. The first attempt to prepare a Slovenian opisthobranch catalogue was made by Turk (2000), who presented a checklist based on record and photographs made during his SCUBA diving excursions. In that particular work, the author presented a survey of opisthobranch fauna of the Adriatic Sea with particular reference to Slovenian coastal waters. Five years later, the same author (Turk, 2005a, b) reported on the very first record of nudibranch *Cumanotus beaumonti* for the Mediterranean sea.

Recent developments in underwater sampling techniques have improved considerably the knowledge of marine biodiversity in many areas of the Mediterranean Sea. Through use of *in vivo* sampling of marine fauna with non-destructive techniques, an impressive number of new species for the Slovenian coastal sea has been detected. Among them, some opisthobranch species have also been recorded.

The aim of this work is to present some new data regarding the opisthobranch molluscs in the Slovenian coastal sea.

## MATERIAL AND METHODS

## Study area

Slovenian coastal waters are located in the southern part of the Gulf of Trieste. The Gulf covers approximately 600 km<sup>2</sup> with a water volume of about 9.5 km<sup>3</sup>. It is a shallow semi-enclosed gulf influenced by freshwater inflow, bottom sediment resuspensions and different sources of pollution. The Slovenian coastline is approximately 46 km long and was once composed exclusively of sandstone (flysch), which is the major source of detrital material. The maximum depth of 33 m was measured in a depression close to Piran.

The area is characterized by high habitat type diversity. The shallower areas are covered by the biocoenosis of photophilous algae, characterized by the association *Cystoseiretum crinitum*, and sea grass meadows of *Cymodocea nodosa*. The samplings were performed also in a sea grass meadow of *Posidonia oceanica*, situated

between the coastal towns of Izola and Koper. The deeper areas are characterized by sandy and muddy habitat types.

## Material and methods

The collection of opisthobranch molluscs is fairly difficult. Although the majority of species are characterized by vivid aposematic coloration, sea slugs are normally small-sized animals, present in low densities and usually inhabiting peculiar habitat types. The information of opisthobranch fauna, which is the basis of the present work, was gathered with different sampling methods and approaches. The majority of data originate from:

- (i) visual transects: during the decade from 1998 to 2008, numerous marine biodiversity sampling have been performed in Slovenian coastal waters (Lipej et al., 2003);
- (ii) selective samplings: the collection of opisthobranch data was particularly intense during the sampling for the BSc Thesis by one of the authors (S. Šamu) in the period from July 2003 to September 2004;
- (iii) dredging: few specimens have been recorded by sampling ichthyofauna with special dredge adopted for sampling in seagrass meadows;
- (iv) random samplings: many opisthobranch molluscs have been detected accidentally by studying other components of biodiversity;
- (v) underwater photography: many specimens have been occasionally photographed.

Sea slugs have been identified by the use of different identification keys such as Barletta (1980) and Schmekel & Portmann (1982). The photographic monograph of opisthobranchs by Trainito (2005) was very helpful for identification based solely on photographs. For certain species, the web sites [www.seaslugforum](http://www.seaslugforum) (SSF) and [www.medsnugs.de](http://www.medsnugs.de) were checked and consulted. The photographs of the majority of specimens, which had previously not been reported by Turk (2000) for the Slovenian part of the Adriatic Sea in his checklist, were included in this paper (Plates I–III). The taxonomic categories are arranged according to SSF. Within each family, genera (and species) were arranged in alphabetical order.

## RESULTS

## Subclassis OPISTHOBRANCHIA

## Order CEPHALASPIDEA

## Superfamily Philinoidea

## Family Philidinidae

*Philine aperta* (Linne, 1767)

The only specimen of this species was recorded on May 23, 2003, in a sample taken by benthic dredge. The

sampling was performed on a circalittoral soft bottom at a depth of 22 m, close to the sewage outfall in waters off Piran. This species has been reported by De Min & Vio (1997) for the Slovenian coastal sea as a rare species, present mainly in the Strunjan Nature Reserve. Vio & De Min (1999) referred to this species as occurring along the Istrian coast near Črvar.

**Superfamily Runcinoidea**  
**Family Runcinidae**

***Runcina adriatica* Thompson, 1980**

This tiny, only few mm long sea slug was found in the mediolittoral sand in front of the Marine Biology Station in Piran (MBS) on January 26, 2006 (Fig. 1).

**Order SACOGLOSSA**  
**Superfamily Elysioidea**  
**Family Elysiidae**

***Elysia timida* (Risso, 1818)**

*E. timida* (Fig. 2) is amongst the most common and abundant sea slugs in the studied area. This herbivorous sea slug was found in most of the studied areas, particularly in the shallow water from 1 to 5 m depth on rocks, covered with algal turf.

***Elysia viridis* (Montagu, 1804)**

*E. viridis* is fairly rare in comparison with the previous species, considering that only two specimens have been recorded. The first was found in the nest of the wrasse *Symphodus roissali*, built by different algal remains within Debeli rtič Natural Monument at a depth of 1.2 m. The second specimen has been found crawling on *Cystoseira barbata* in the area between Fiesa and Pačug at a depth of 4 m (Fig. 3).

***Elysia* cf. *gordanae* Thompson & Jaklin, 1988**

A specimen of this elysiid sea slug (Fig. 4) has been found in front of the MBS on March 26, 2005. Due to the diagnostic features such as rather low parapodia (Thompson & Jaklin, 1988), the specimen could be identified as the recently discovered elysiid *E. gordanae*.

***Thuridilla hopei* (Verany, 1853)**

This species was also very abundant at many stations off the Slovenian coast. *T. hopei* was recorded in the depth range from 1 to 12 m. It inhabits different microhabitat types in the biocoenosis of photophilous algae.

**Superfamily Limapontioidea**  
**Family Limapontiidae**

***Ercolania coerulea* Trinchese, 1892**

A single specimen of this species was recorded on March 26, 2005, in front of the MBS. It was found in the

upper rocky infralittoral with patches of sand and gravel at 3 m depth (Fig. 5).

**Order ANASPIDEA**  
**Superfamily Aplysioidea**  
**Family Aplysiidae**

***Aplysia fasciata* Poiret, 1789**

On December 6, 2006, a 250 mm long sea hare was found stranded in front of the MBS. The specimen was still alive, and was therefore placed in the aquarium. It was completely black in colour.

***Aplysia punctata* Cuvier, 1803**

*Aplysia punctata* is a very common sea slug, occurring mostly in spring months when gathering in large numbers. It was recorded in the biocoenosis of photophilous algae in the majority of such localities. It seems to prefer the rocky bottom with a dense algal belt in the depth range from 2 to 4 m. The observed specimens ranged from 50 to 80 mm in size and were very variable in colour pattern.

***Bursatella leachii* de Blainville, 1817**

The non-indigenous sea slug *B. leachi* (Fig. 6) was found on numerous occasions in the studied area. It was found already in 2001 in front of the MBS as well as two years later (November 27, 2003) at the very same location. During the survey of cryptobenthic species, *B. leachi* was found on the algae *Padina pavonica* at a depth of 2.2 m at Seča on October 9, 2006. The majority of data originate from 2007. In March 2007, two specimens were recorded in the Piran harbour in front of the Piran Aquarium. A specimen was also recorded in a fish cage in the waters off Sečovlje in August 2007.

On October 1, 2007, an immense density of specimens of this very species was observed in a saltpan channel at Strunjan. At a depth lower than 1 m, approximately 50 sea slugs were counted in an area of a square meter. On January 9, 2008, a single specimen was found in the material, sampled by a special seagrass meadow dredge in the sea grass meadow of *Cymodocea nodosa* in Strunjan at a depth of 2 m.

**Order NOTASPIDEA**  
**Superfamily Pleurobrancoidea**  
**Family Pleurobranchidae**

***Berthella ocellata* (Delle Chiaje, 1828)**

A specimen of this species was documented at Fiesa on a rocky bottom on July 6, 2003. It was found among algae on big rocks at a depth of 7 m. Another specimen was found in July 2003 under rocks at Cape Ronek at a depth of 5 m. This species had been previously recorded by Turk (2000) at Piran, where a specimen was found under stones at 1 m depth.

**Order NUDIBRANCHIA****Suborder DORIDINA****Familij Goniodorididae*****Trapania lineata* Haefelfinger, 1960**

A single specimen of this species (Fig. 7) was found on March 19, 2008, with ascidian *Phallusia mammilata* on the muddy bottom at 8 m depth in front of MBS.

***Trapania maculata* Haefelfinger, 1960**

Four records of this species have been documented. Firstly, the species was recorded on the black sponge *Cacospongia scalaris* at a depth of 3.5 m in front of MBS. Two specimens of *T. maculata* were found on January 21, 2008, again in front of MBS. Another specimen of this species was registered on muddy bottom at a depth of 8.8 m at the very same locality on March 30, 2008.

**Family Onchidorididae*****Onchidoris neapolitana* (Delle Chiaje, 1841)**

On February 27, 2008, a red coloured sea slug was observed on a bryozoan covered stone in front of MBS (Fig. 8). It occurred at a depth of ca. 4 m in the sea grass meadow of *Cymodocea nodosa*. The specimen was about 5 mm in total length. The species was determined as *O. neapolitana*, and B. Rudman, after consulting the Sea slug forum, subsequently confirmed this fact.

**Family Dorididae*****Archidoris pseudoargus* (von Rapp, 1827)**

A specimen of this species was recorded on March 26, 2005, in front of MBS. It was found on rocks in a biocoenosis of photophilic algae at 3 m depth. Since a plethora of photographs of specimens more or less similar to this species were taken in the studied period, the species is probably common.

***Discodoris rosi* Ortea, 1979**

A red dorid sea slug was registered in April 2005 in front of MBS. It was found in phytal zone with muddy patches at a depth of app. 6 m. It was determined as *D. rosi*. The large white rings over the mantle are less evident (Fig. 9).

***Doris cf. berthelotti* (d'Orbigny, 1839)**

At four localities (NM Punta Madonna Piran, Vila Tartini at Strunjan, Fiesa, Belveder), ten specimens of this species were recorded during different seasons. They were found at a depth ranging from 2 to 10 m in different habitats such as mud, sand and rocky bottom. This species had previously been recorded at Fiesa by Turk (2000).

***Geitodoris planata* (Alder & Hancock, 1846)**

The only specimen of this species (Fig. 10) was observed and subsequently photographed in December 2005 in front of MBS. It was recorded under stones at a depth of 1 m. There is still much confusion in the modern scientific literature about the discrimination between *G. planata* and *Discodoris stellifera*. Both have star-shaped pattern on the back, and can be reliably distinguished only on the basis of radula. According to Rudman (2005b), this specimen should be considered as *Geitodoris planata*.

***Jorunna tomentosa* (Cuvier, 1804)**

A specimen of this species, crawling on a sponge *Chondrilla nucula*, was photographed (Fig. 11) in April 2005 in front of MBS. The dense coverage of caryophyllidia gives the species the velvety form.

***Rostanga rubra* (Risso, 1818)**

The only specimen was recorded and photographed (Fig. 12) in April 2005 in front of MBS in the upper infralittoral (3 m depth). In view of the whitish line between the rhinophore pockets, the species has been determined as *R. rubra*.

**Family Chromodorididae*****Chromodoris krohni* (Vérany, 1846)**

This species (Fig. 13) was recorded on several occasions in July and August 2003 and in summer 2008. It was found mainly at depths ranging from 2 to 7 m in a shady, precoralligenous aspect of the coralligenous biocoenosis. In shallower waters (below 3 m), it can be found under stones.

***Chromodoris luteorosea* (Rapp, 1827)**

*Chromodoris luteorosea* was recorded in the area on several occasions. The first specimen was found on August 1, 2000, at Cape Madonna Natural Monument in Piran at 7 m depth in a precoralligenous aspect. Another specimen of this species was documented on October 2, 2006, at a depth of 1.8 m close to the wreck of the ship Rex along the coastline between Izola and Koper. It was found on a stone covered with turf. The third specimen was found on July 19, 2007, in the sea grass meadow of *Posidonia oceanica* between Koper and Izola. The fourth specimen was recorded and photographed in front of MBS on March 12, 2008. It was found in muddy habitat at a depth 3.8 m. In the summer 2008, the species was recorded very frequently at almost every studied site.

***Chromodoris purpurea* (Risso in Guérin, 1831)**

A single specimen of *C. purpurea* (Fig. 14) was found on June 17, 2008, on a stone in the sea grass meadow of *Cymodocea nodosa* in the area of Sv. Jernej close to Debeli rtič Natural monument.

***Hypselodoris orsinii* (Verany, 1846)**

This hypselodorid sea slug (Fig. 15) was recorded on two occasions only. The first record originates from June 7, 2003, the second from July 2, 2007. In both cases, specimens were found on sponges. It is much smaller than other species of this genus. Although the taxonomical differentiation among species of this genus is somewhat difficult, the dorsal pattern of the observed specimens is so typical that we were able to identify this species as *H. orsinii*.

***Hypselodoris* cf. *tricolor* (Cantraine, 1835)**

Many specimens of this species were recorded in the studied area in different types of rocky bottom. Due to the similarity with other species of the genus *Hypselodoris*, it is still fairly difficult to determine the species with utmost certainty. However, comparing the photographs with the photographic documentation of identification keys (e.g. Trainito, 2005) and photographic evidence in the [www.seaslugforum](http://www.seaslugforum), our specimens' diagnostic features fit close to the *H. tricolor*.

***Hypselodoris villafranca* (Risso, 1818)**

This species is quite common in the Slovenian part of the Gulf of Trieste. Three specimens of this species were recorded on September 9, 2003, in the waters off Villa Tartini at Strunjan. They were found under stones in a sandy environment. Other records originate from samplings carried out in 2008, where few specimens were photographed in seagrass meadow of *Cymodocea nodosa* in front of MBP on February 27, March 12, and March 19, 2008.

**Family Dendrodorididae*****Dendrodoris grandiflora* Rapp, 1827**

A specimen of *D. grandiflora* was found on June 8, 2008, at Fiesa. It was recorded while crawling on a stone in a rocky habitat of the biocoenosis of photophilous algae at a depth of 3 m.

***Dendrodoris limbata* (Cuvier, 1804)**

As a fairly common sea slug, *Dendrodoris limbata* was found at different localities of Slovenian coastal waters. It was recorded at depths ranging from 1 to 8 m. The majority of specimens were hidden under bare stones, while others were found in sandy habitat. They were found in three colour varieties; brown with a yellow notum edge margin, black with a pale notum edge, and a paler variety with many dark patches (Fig. 16).

**Suborder DENDRONOTINA****Family Tethydidae*****Tethys fimbria* Linne, 1767**

This large opisthobranch was recorded on May 23, 2003, in a sample taken by benthic dredge. The sampling was performed on soft bottom at a depth of 22 m, close to the sewage outfall in the waters off Piran. A specimen of *T. fimbria* was photographed in front of MBS on February 21, 2004. The specimen was found crawling in a muddy habitat type at the depth of 26 m.

**Suborder AEOLIDINA****Family Flabellinidae*****Flabellina affinis* (Gmelin, 1791)**

Four specimens of this species were recorded in July and September 2003 at Cape Madona Natural Monument and between Fiesa and Pacug. All specimens were found on hydroid colonies of *Eudendrium* sp. at depths ranging between 9 and 12 m.

***Flabellina ischitana* Hirano & Thompson, 1990**

This species quite resemble the species above, except that the cerata are of distinct orange or red colour. It is a fairly frequent species, occurring in huge numbers at depths ranging from 10 to 12 m at different localities. Generally they were found grazing on colonial hydroid *Eudendrium* sp.

***Flabellina pedata* (Montagu, 1815)**

This species was recorded twice. One specimen was registered on September 4, 2003, in the southern part of Cape Madona Natural monument at a depth of 9.3 m, and the second on a precoralligenous rock in waters off the northern coastline of Piran at 11.1 m depth.

**Family Cumanotidae*****Cumanotus beaumonti* (Eliot, 1906)**

In April 2005, two specimens of this species were recorded at Cape Madona Natural Monument. They were found on a muddy bottom at a depth of 20 m. This was the very first record of such species in the Mediterranean Sea (Turk, 2005a, b). It had previously been reported only from the British Isles and Norway, where it is considered very rare (Rudman, 2005a, b). Afterward it was found also in the waters off Ravenna in the western Adriatic (Magnani, 2006).

In March 2008, a specimen of *C. beaumonti* was found in muddy habitat at a depth of 10 m in front of MBS. It was registered on athecate solitary hydroid *Corymophra nutans* (Fig. 17).

**Family Eubranchiidae*****Eubranchus farrani* (Alder & Hancock, 1844)**

A specimen of this species (Fig. 18) was recorded in front of MBS in March 2006. It was found in a dense algal belt of *Cystoseira barbata* in the biocoenosis of photophilic algae at a depth of 2 m.

**Family Aeolidiidae*****Berghia coerulescens* (Laurillard, 1830)**

Four specimens of *B. coerulescens* were recorded. The first was found on June 11, 2003, in the algal belt at 5.5 m depth. The second specimen was recorded on precoralligenous aspect or community at 11.4 m in the waters off the northern coast of Piran in September 2003. The third specimen was recorded on March 26, 2005, in front of MBS. The fourth specimen was recorded on July 17, 2008, at 2.5 m depth in the algal belt of *Cystoseira barbata* in the waters along the boundary of Cape Madona Natural Monument in Piran. This species is probably much more common than evident from the samplings. It had previously been recorded also by Turk (2000) at Strunjan.

***Spurilla neapolitana* (Delle Chiaje, 1823)**

Two specimens were found on November 3, 2005, within the culture of clams (*Mytilus galloprovincialis*) at the mariculture platform in Piran Bay. Another specimen was photographed at the very same site on March 2, 2007. According to Turk (2000), this species is considered a quite common sea slug inhabiting shallow waters.

**Family Glaucidae*****Crataena peregrina* (Gmelin, 1791):**

This sea slug is one of the commonest opisthobranch species in the area. The great majority of *C. peregrina* were found to graze the hydrozoan colonies of *Eudendrium* sp. on a precoralligenous belt and solitary rocks on the sandy bottom. They were found at depths ranging from 3 to 12 m.

***Dondice banyulensis* Portmann & Sandmaier, 1960**

A specimen of this species (Fig. 19) was recorded in March 2006 just in front of MBS. It was found in a dense algal belt of *Cystoseira barbata* in a biocoenosis of photophilic algae at 2 m depth.

***Facelina bostoniensis* (Couthouy, 1838)**

The specimen of *F. bostoniensis* (Fig. 20) was recorded on a single occasion in spring 2003 just off MBS. It was recorded on pebbles at 1 m depth in the biocoenosis of photophilous algae (*Cystoseiretum criinitae*).

***Facelina fusca* Schmekel, 1966**

The only specimen of *F. fusca* (Fig. 21) was recorded on September 4, 2008, on rocky bottom with the sponge *Verongia aerophoba* at 6.9 m depth at Pacug. The sea slug has been preserved in our collection. It highly resembles the specimen photographed in the waters off Trieste (Turk, 2005c), tentatively determined by Rudman (2005c) as *F. fusca*.

**DISCUSSION**

In the present work, we are reporting on 40 species of sea slugs recorded in Slovenian coastal waters during the last decade. Taking into account the recent malacological surveys, performed by other authors (De Min & Vio, 1997; Turk, 2000, 2005a, b) in the Slovenian part of the Gulf of Trieste, altogether 66 opisthobranch molluscs have been recorded. If the entire Gulf of Trieste is considered, then the complete number is at least 75 species (Tab. 1). Among 66 species, more than 78% (52 species) are represented by orders Cephalaspidea and Nudibranchia (Tab. 2). In a previous report, Turk (2000) listed 23 opisthobranch species from the very same area in Slovenian part of the Gulf of Trieste. We failed to record 7 of the species mentioned in his checklist, but we have completed the list with 14 species previously not reported, together with *Cumanotus beaumonti*, recorded for the very first time by Turk (2005a, b).

In the Mediterranean Sea, some 400 species of Opisthobranchia were recorded (Cattaneo-Vietti & Thompson, 1989) with an increasing trend, mainly due to the increasing number of reported alien species (Daskos & Zenetos, 2007). The obtained data for the Slovenian part of the Adriatic Sea suggest that only a small portion (16.5%) of opisthobranchs inhabit the studied area. However, as we have found certain doriid species, which we were not able to identify (and are therefore not presented in this manuscript), the obtained number would certainly increase in the nearby future with the solving of actual taxonomical problems. Since we have studied only relatively low number of habitat types and localities as well, the list of sea slugs of the Slovenian part of the Gulf of Trieste is to our opinion still far from complete.

**Tab. 1: Survey of opisthobranch molluscs found in the Italian (ITA) and Slovenian (SLO) parts of the Gulf of Trieste. Legend: 1 – Vio & de Min (1996), 2 – De Min & Vio (1997), 3 – Turk (2000) and 4 – this paper.**

**Tab. 1: Pregled polžev zaškrjarjev (Opisthobranchia) v slovenskem (SLO) in italijanskem (ITA) delu Tržaškega zaliva. Legenda: 1 – Vio & de Min (1996), 2 – De Min & Vio (1997), 3 – Turk (2000) in 4 – to delo.**

No.	Species	Order	Vio & De Min (1996)	De Min & Vio (1997)	Turk (2000)	this work
			ITA	SLO	SLO	SLO
1	<i>Acteon tornatilis</i>	Cephalaspidea	+	+		
2	<i>Retusa mammilata</i>	Cephalaspidea	+	+		
3	<i>Retusa leptoneilema</i>	Cephalaspidea	+			
4	<i>Retusa semisulcata</i>	Cephalaspidea	+			
5	<i>Retusa obtusa</i>	Cephalaspidea		+		
6	<i>Retusa truncatula</i>	Cephalaspidea	+	+		
7	<i>Cylichnina laevisculpta</i>	Cephalaspidea	+	+		
8	<i>Cylichnina multiquadrata</i>	Cephalaspidea		+		
9	<i>Cylichnina umbilicata</i>	Cephalaspidea	+	+		
10	<i>Volvulella acuminata</i>	Cephalaspidea	+	+		
11	<i>Ringicula auriculata</i>	Cephalaspidea	+	+		
12	<i>Bulla striata</i>	Cephalaspidea	+	+		
13	<i>Haminoea hydatis</i>	Cephalaspidea	+	+		
14	<i>Haminoea navicula</i>	Cephalaspidea	+	+		
15	<i>Atys jeffreysi</i>	Cephalaspidea	+	+		
16	<i>Weinkauffia turgidula</i>	Cephalaspidea	+	+		
17	<i>Philinopsis depicta</i>	Cephalaspidea	+		+	
18	<i>Philine aperta</i>	Cephalaspidea	+	+		+
19	<i>Philine catena</i>	Cephalaspidea		+		
20	<i>Philine scabra</i>	Cephalaspidea	+			
21	<i>Laona pruinosa</i>	Cephalaspidea	+			
22	<i>Cylichna crosseii</i>	Cephalaspidea	+			
23	<i>Cylichna cylindracea</i>	Cephalaspidea	+	+		
24	<i>Roxania utriculus</i>	Cephalaspidea	+			
25	<i>Scaphander lignarius</i>	Cephalaspidea	+	+		
26	<i>Runcina adriatica</i>	Cephalaspidea				+
27	<i>Creseis acicula</i>	Thecosomata	+	+		
28	<i>Pleurobranchus membranaceus</i>	Notaspidea	+			
29	<i>Berthella stellata</i>	Notaspidea			+	
30	<i>Berthella aurantiaca</i>	Notaspidea	+			
31	<i>Berthella ocellata</i>	Notaspidea			+	+
32	<i>Elysia timida</i>	Sacoglossa				+
33	<i>Elysia viridis</i>	Sacoglossa				+
34	<i>Elysia</i> cf. <i>gordanae</i>	Sacoglossa				+
35	<i>Thuridilla hopei</i>	Sacoglossa			+	+

No.	Species	Order	Vio & De Min (1996)	De Min & Vio (1997)	Turk (2000)	this work
			ITA	SLO	SLO	SLO
36	<i>Boselia mimetica</i>	Sacoglossa			+	
37	<i>Calliopaea bellula</i>	Sacoglossa			+	
38	<i>Ercolania coerulea</i>	Sacoglossa				+
39	<i>Akera bullata</i>	Anaspidea	+	+		
40	<i>Aplysia punctata</i>	Anaspidea			+	+
41	<i>Aplysia depilans</i>	Anaspidea	+	+		
42	<i>Aplysia fasciata</i>	Anaspidea	+	+		+
43	<i>Bursatella leachii</i>	Anaspidea	+			+
44	<i>Trapania lineata</i>	Nudibranchia				+
45	<i>Trapania maculata</i>	Nudibranchia			+	+
46	<i>Doris</i> cf. <i>berthelotti</i>	Nudibranchia			+	+
47	<i>Archidoris pseudoargus</i>	Nudibranchia			+	+
48	<i>Jorunna tomentosa</i>	Nudibranchia				+
49	<i>Rostanga rubra</i>	Nudibranchia				+
50	<i>Onchidoris neapolitana</i>	Nudibranchia				+
51	<i>Geitodoris planata</i>	Nudibranchia				+
52	<i>Chromodoris krohni</i>	Nudibranchia				+
53	<i>Chromodoris luteorosea</i>	Nudibranchia			+	+
54	<i>Chromodoris purpurea</i>	Nudibranchia				+
55	<i>Hypselodoris</i> cf. <i>tricolor</i>	Nudibranchia			+	+
56	<i>Hypselodoris orsinii</i>	Nudibranchia				+
57	<i>Hypselodoris villafranca</i>	Nudibranchia				+
58	<i>Platydoris argo</i>	Nudibranchia			+	
59	<i>Discodoris rosi</i>	Nudibranchia				+
60	<i>Dendrodoris grandiflora</i>	Nudibranchia			+	+
61	<i>Dendrodoris limbata</i>	Nudibranchia			+	+
62	<i>Tethys fimbria</i>	Nudibranchia			+	+
63	<i>Cumanotus beaumonti</i>	Nudibranchia				+
64	<i>Eubranchius farrani</i>	Nudibranchia				+
65	<i>Facelina bostoniensis</i>	Nudibranchia				+
66	<i>Facelina fusca</i>	Nudibranchia				+
67	<i>Flabellina affinis</i>	Nudibranchia			+	+
68	<i>Flabellina ischitana</i>	Nudibranchia			+	+
69	<i>Flabellina pedata</i>	Nudibranchia			+	+
70	<i>Berghia verrucicornis</i>	Nudibranchia			+	
71	<i>Berghia coerulescens</i>	Nudibranchia			+	+
72	<i>Spurilla neapolitana</i>	Nudibranchia			+	+
73	<i>Cratena peregrina</i>	Nudibranchia			+	+
74	<i>Dondice banyulensis</i>	Nudibranchia				+
	<b>Total</b>		<b>29</b>	<b>22</b>	<b>23</b>	<b>40</b>

**Tab. 2: The structure of opisthobranch fauna in Slovenian waters and in the entire Gulf of Trieste in terms of different orders. See Table 1 for basic data.**

**Tab. 2: Število vrst v posameznih redovih polžev zaškrGARJEV v slovenskih vodah in v celotnem delu Tržaškega zaliva. Glej Tabelo 1 za osnovne podatke.**

Opisthobranch orders	Slovenian waters	Gulf of Trieste
CEPHALASPIDEA	20	26
SACOGLOSSA	7	7
THECOSOMATA	1	1
NOTASPIDEA	2	4
ANASPIDEA	5	5
NUDIBRANCHIA	31	31
<b>Total</b>	<b>66</b>	<b>74</b>

The majority of species have been recorded only in sporadic cases, while only few species have been found to be relatively common. Among the later we should mention the species such as *Elysia timida*, *Thuridilla hopei*, *Aplysia punctata*, *Flabellina ischitana*, *Cratena peregrina* and *Hypselodoris villafranca*.

Sampling success is related to different factors, affecting the record of sea slugs. Perhaps the major factor is detectability. Many small species are regularly not detected while performing visual census techniques. The detectability is on the other hand related to the presence of a proper habitat type on a small scale and typical bio-coenosis on a larger scale. Certain zoophagous species are in fact related to specific environment, so their occurrence is affected by the occurrence of proper habitat type in the studied area. On the other hand, certain species such as *Discodoris atromaculata*, which is related to a specific host species, the sponge *Petrosia ficiformis*, was not detected, although this sponge has been found at many sites of the studied area. To our opinion, some species will be recorded in the future while performing

sampling in interstitial habitats. Finally, there are some problems that are related to taxonomy itself. In fact, the taxonomy of certain genera is still rather confusing as it is in the case of genus *Hypselodoris* or many doriid species.

The underwater photography is playing a very important role. Many divers are attracted by the aposematic colour of sea slugs, thus a lot of information could be obtained in this way. The case of *Cumanotus beaumonti* is very informative in that regard. Another interesting species, which has been to date recorded only a couple of times, is *Elysia gordanae*. This elysiid species, recently described by Thompson & Jaklin (1988) in adjacent waters off Rovinj (Croatia), has been to date recorded only few times in the Adriatic Sea and in waters off Spain.

Up to date, only one non-indigenous species, *Bursatella leachi*, has been recorded in Slovenian coastal waters. Since at some sites the density of *B. leachi* reached the impressive number of 50 individuals per square meter, this species should be considered as an established one. This species has been documented already by Jaklin & Vio (1989) and eventually by De Min & Vio (1997, 1998). It is considered to be a lessepsian migrant, originating from the Indian Ocean.

#### ACKNOWLEDGMENTS

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# POLŽI ZAŠKRGARJI (MOLLUSCA: GASTROPODA OPISTHOBRANCHIA) IZ SLOVENSKEGA OBREŽNEGA MORJA (SEVERNI JADRAN)

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## POVZETEK

Avtorji poročajo o seznamu golih polžev in sorodstva iz skupine polžev zaškrjarjev *Opisthobranchia*, ki nase-ljujejo slovenski del Jadranskega morja. Z različnimi metodami so popisali 40 vrst. Seznam vseh doslej ugotovljenih vrst na podlagi pričujočega vzorčevanja in dosedaj objavljenih pregledov malakofavne preučevanega območja šteje najmanj 66 vrst za slovenski del in skupaj 75 vrst za celotni del Tržaškega zaliva. Med ugotovljenimi vrstami je tudi tujerodna vrsta *Bursatella leachi*. Večina vrst je bila ugotovljena v nekaj primerih in le za nekaj vrst velja, da so v danem območju pogoste. Glede na dejstvo, da so vzorčevali na manjšem številu habitatnih tipov, avtorji pričakujejo, da se bo v prihodnosti število ugotovljenih vrst še povečalo.

**Ključne besede:** polži zaškrjarji, seznam vrst, Slovenija, Tržaški zaliv, Jadransko morje

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**PLATES I–III: Some opisthobranch species, recorded in the Slovenian sea.**

**TABLE I–III: Nekateri polži zaškrgarji, ugotovljeni v slovenskem morju.**

Photographs credits / Avtorji fotografij: BM – Borut Mavrič, LL – Lovrenc Lipej, SA – Samo Alajbegović, TM – Tihomir Makovec, ŽD – Žiga Dobrajc.

**PLATE I/ TABLA I**

**Fig. 1/Sl. 1:** *Runcina adriatica* (BM)

**Fig. 2/Sl. 2:** *Elysia timida* (LL)

**Fig. 3/Sl. 3:** *Elysia viridis* (SA)

**Fig. 4/Sl. 4:** *Elysia cf. gordanae* (TM)

**Fig. 5/Sl. 5:** *Ercolania coerulea* (SA)

**Fig. 6/Sl. 6:** *Bursatella leachii* (BM)

**Fig. 7/Sl. 7:** *Trapania lineata* (BM)

**Fig. 8/Sl. 8:** *Onchidoris neapolitana* (BM)

**PLATE II/ TABLA II**

**Fig. 9/Sl. 9:** *Discodoris rosi* (TM)

**Fig. 10/Sl. 10:** *Geitodoris planata* (SA)

**Fig. 11/Sl. 11:** *Jorunna tomentosa* (TM)

**Fig. 12/Sl. 12:** *Rostanga rubra* (TM)

**Fig. 13/Sl. 13:** *Chromodoris krohni* (LL)

**Fig. 14/Sl. 14:** *Chromodoris purpurea* (ŽD)

**Fig. 15/Sl. 15:** *Hypselodoris orsinii* (TM)

**Fig. 16/Sl. 16:** *Dendrodoris limbata* (SA)

**PLATE III/ TABLA III**

**Fig. 17/Sl. 17:** *Cumanotus beaumonti* (ŽD)

**Fig. 18/Sl. 18:** *Eubranchius farrani* (BM)

**Fig. 19/Sl. 19:** *Dondice banyulensis* (BM)

**Fig. 20/Sl. 20:** *Facelina bostoniensis* (TM)

**Fig. 21/Sl. 21:** *Facelina fusca* (LL)

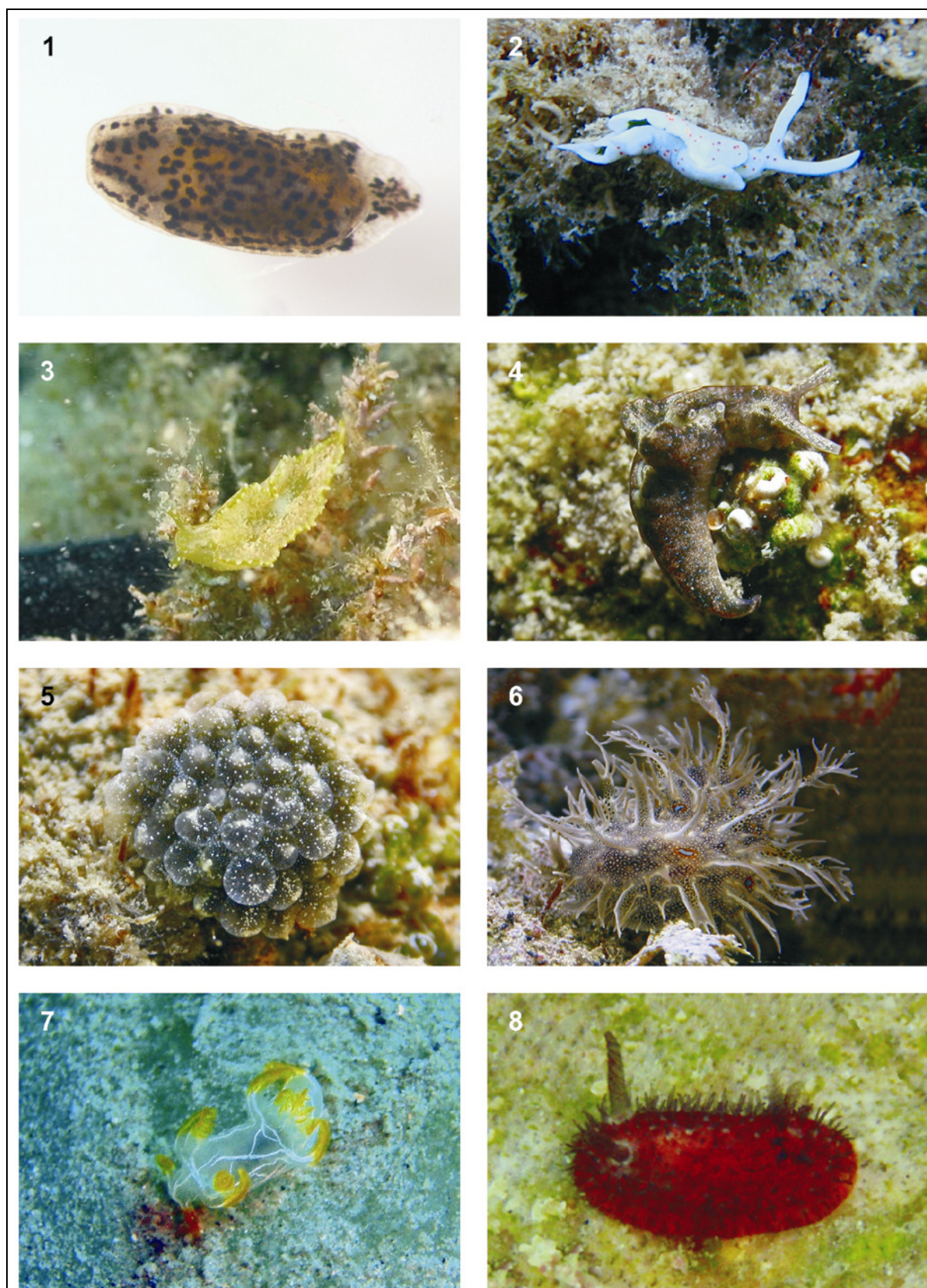


PLATE I/ TABLA I

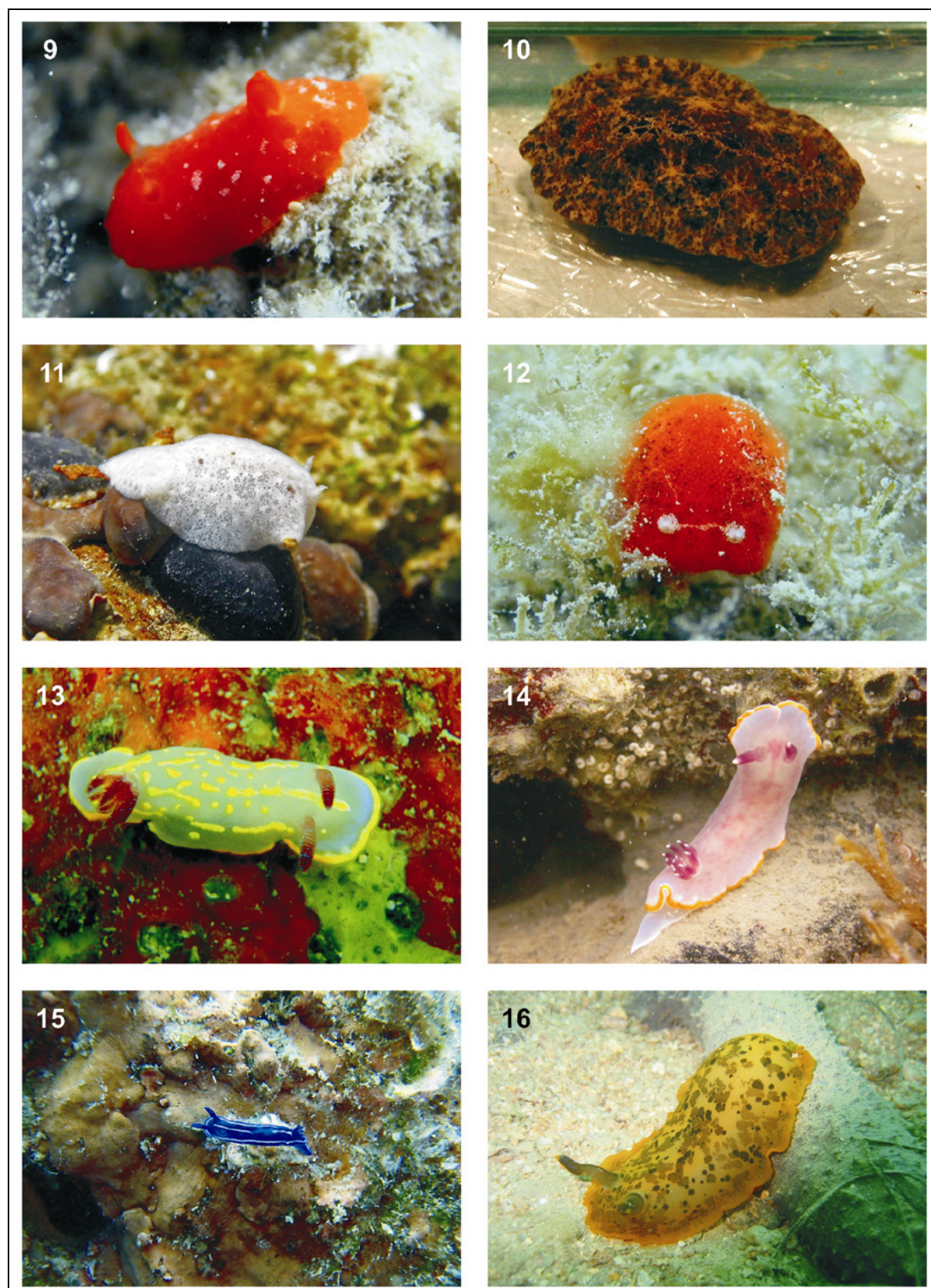


PLATE II/ TABLA II

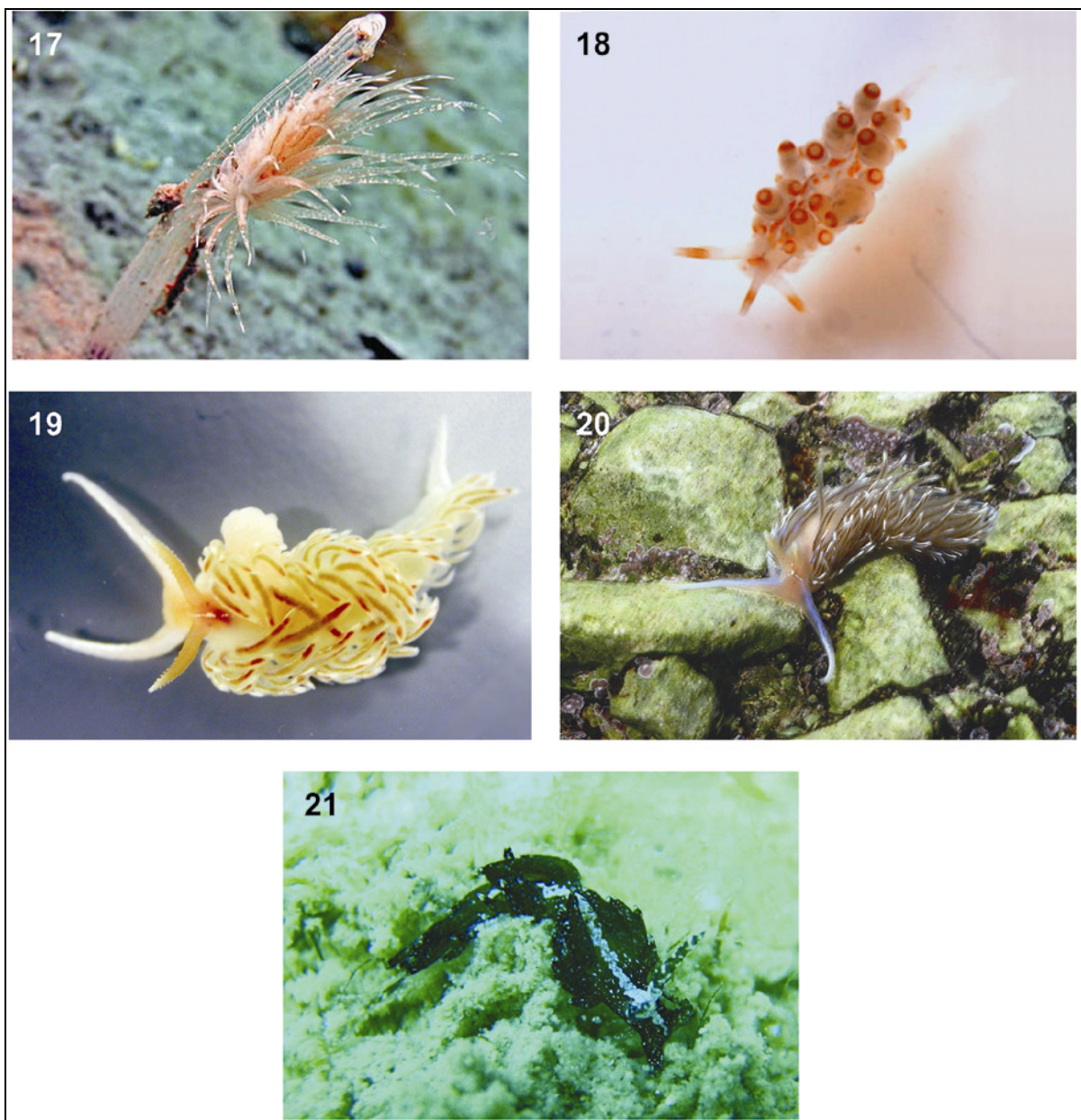


PLATE III/ TABLA III

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## BOTTLENOSE DOLPHINS (*TURSIOPS TRUNCATUS*) IN SLOVENIAN AND ADJACENT WATERS (NORTHERN ADRIATIC SEA)

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### ABSTRACT

A local population of bottlenose dolphins (*Tursiops truncatus*) in Slovenian and adjacent waters (northern Adriatic Sea) was studied between 2002 and 2008. Boat-based surveys, land-based surveys and standard photo-identification procedures were carried out. A total of 120 sightings were recorded and 101 well-marked dolphins photo-identified. Resighting rates within and between years showed a relatively high rate of site fidelity for some individuals. The group size ranged from 1 to 43. Offspring were present in 53.3% of the groups. Annual mark-recapture density estimates of 0.069 dolphins/km<sup>2</sup> seem to be good baseline information for conservation management.

**Key words:** bottlenose dolphin, *Tursiops truncatus*, ecology, photo-identification, Slovenia, northern Adriatic

## TURSIOPPI (*TURSIOPS TRUNCATUS*) IN ACQUE SLOVENE E ADIACENTI (ADRIATICO SETTENTRIONALE)

### SINTESI

Una popolazione locale di tursiopi (*Tursiops truncatus*) in acque slovene e adiacenti (Adriatico settentrionale) è stata studiata nel periodo dal 2002 al 2008. La ricerca è stata condotta tramite avvistamenti da imbarcazioni e da stazioni d'osservazione da terra, nonché con le procedure standard di foto-identificazione. In totale sono stati effettuati 120 avvistamenti ed identificati 101 individui. Il tasso di riavvistamento dei singoli delfini nell'arco dell'anno e fra anni diversi indica un alto grado di frequenza di determinati individui nell'area. La grandezza dei gruppi variava da 1 a 43 individui. I cuccioli erano presenti nel 53,3% dei gruppi. La valutazione annuale della densità è pari a 0,069 tursiopi / km<sup>2</sup>, il che rappresenta un'informazione di base importante nella tutela e gestione dei tursiopi.

**Parole chiave:** tursiopi, *Tursiops truncatus*, ecologia, foto-identificazione, Slovenia, Adriatico settentrionale

## INTRODUCTION

The bottlenose dolphin (*Tursiops truncatus*, Montagu, 1821) is one of the best studied cetacean species in the world (Shane *et al.*, 1986; Leatherwood & Reeves, 1990; Connor *et al.*, 2000; Bearzi *et al.*, 2008b) and one of the most common and widespread cetacean species in the Mediterranean Sea (Notarbartolo di Sciarra *et al.*, 1993; Reeves & Notarbartolo di Sciarra, 2006; Bearzi *et al.*, 2008b). Today, the Mediterranean subpopulation is proposed for being listed as "Vulnerable" under IUCN (World Conservation Union) criterion A2d, c, e and its present distribution is considered to be fragmented into units with relatively low densities (Reeves & Notarbartolo di Sciarra, 2006).

No consistent and systematic research on this species had been carried out in Slovenian waters and adjacent waters of Italy and Croatia (Gulf of Trieste and western Istria, northern Adriatic Sea) prior to this study. The only long-term study of free ranging bottlenose dolphins in the Adriatic prior to this study started in 1987 and is being carried out in Kvarnerić, Croatia (Bearzi *et al.*, 1997, 1999; Mackelworth *et al.*, 2003; Fortuna, 2006; Rako, 2006).

The bottlenose dolphin is the only cetacean species regularly observed in the northern Adriatic Sea in the last 20 years (Kryštufek & Lipej, 1993; Notarbartolo di Sciarra *et al.*, 1993; Bearzi & Notarbartolo di Sciarra, 1995; Bearzi *et al.*, 2004).

By studying bottlenose dolphin populations in different areas we can gain insight into their habitat use and the way in which varying environmental factors shape their behaviour and population dynamics (Wilson, 1995). By understanding these processes, we can try to make predictions or generalisations about the ecology of the marine ecosystem as a whole and attempt to evaluate the effects that natural and human-induced changes in the environment might have on the status of the ecosystem and species (Wilson, 1995).

The area where this study was carried out is the northernmost part of both the Adriatic Sea and the Mediterranean Sea as a whole. It is a semi-enclosed and shallow area, shared among three countries (Croatia, Italy and Slovenia). Human activities, such as urbanisation, maritime transport, fishery, mariculture and tourism, are very intense. For these reasons, the area is of particular interest for studying bottlenose dolphins in relation to natural and anthropogenic variations in the ecosystem.

The mainly coastal nature of bottlenose dolphins exposes them to a wide variety of human activities and potential threats. Careful evaluation and consistent monitoring of the status of this species and the extent of

human impacts are therefore essential. This is emphasised by national legislation (e.g. the Nature Conservation Act), international conventions (e.g. the Convention on Migratory Species), European directions and regulations (e.g. the Habitats Directive and Council Regulation (EC) No. 812/2004) and regional conservation agreements, such as the Agreement on the Conservation of Cetaceans of the Black Sea, Mediterranean Sea and Contiguous Atlantic Area (ACCOBAMS), ratified by Croatia, Italy and Slovenia.

This paper presents some preliminary results of a long-term study having been carried out since 2002 to investigate the ecology of bottlenose dolphins inhabiting Slovenian waters and adjacent areas of Croatia and Italy. Part of the data from this research was already presented in various sources (Genov *et al.*, 2004; Genov & Fortuna, 2005; Genov & Wiemann, 2005; Genov & Furlan, 2006). This work is the first attempt to provide data on bottlenose dolphin ecology in this area and baseline information for future studies and effective conservation of this species in the region.

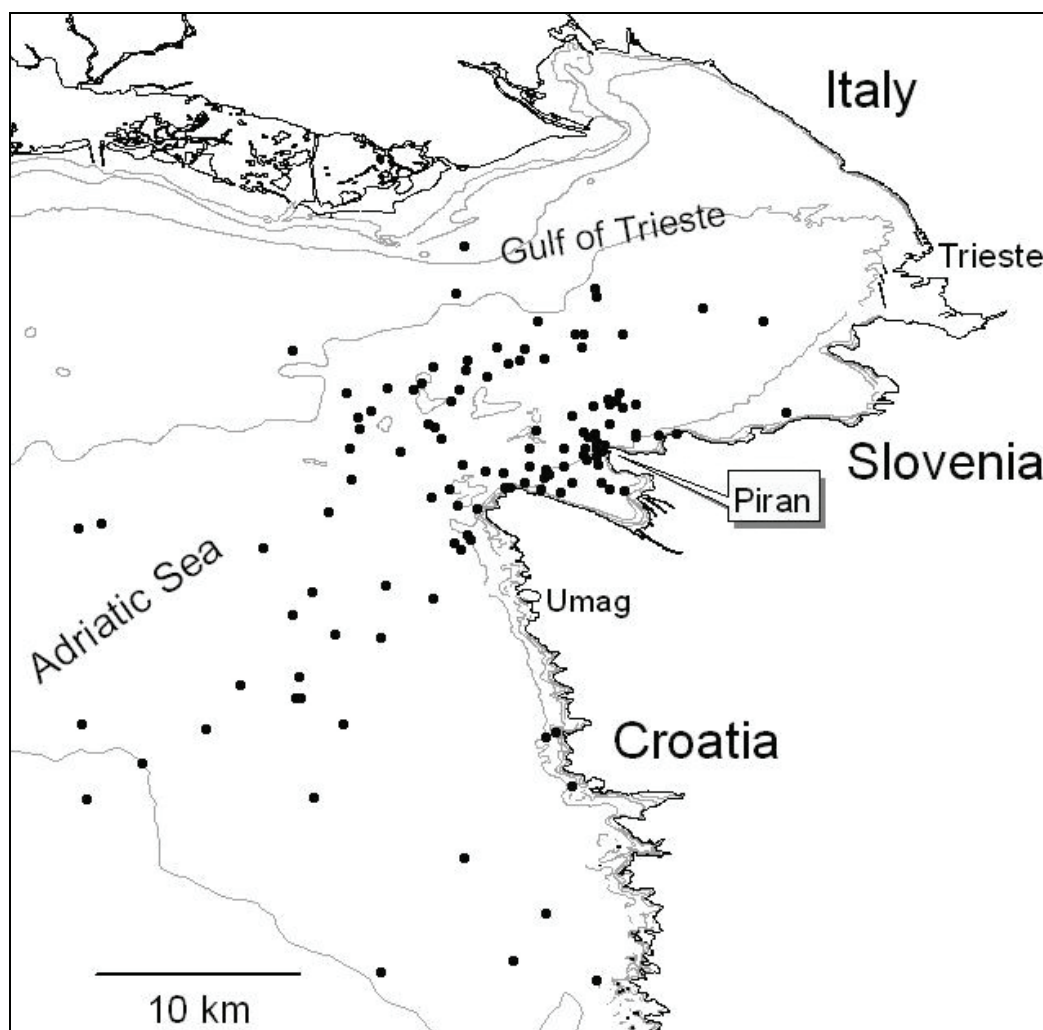
## MATERIAL AND METHODS

## Study area

The whole study area covers roughly 1,200 km<sup>2</sup>, including Slovenian territorial waters, as well as portions of adjacent Italian and Croatian territorial waters of the Gulf of Trieste and the waters off north-western Istria (Fig. 1). The real size of the study area varied between years, due to budgetary and logistic reasons, increasing from about 260 km<sup>2</sup> up to 1,600 km<sup>2</sup>.

This area is mostly characterised by muddy and sandy bottoms, with occasional hard rock bottoms and seagrass meadows of *Posidonia oceanica* and *Cymodocea nodosa* (Lipej *et al.*, 2000). The average depth is 20 m, while the maximum depth is 38 m. The area is inhabited by high biomass benthic communities and characterised by high variations of salinity (32–39 PSU) and water temperature (6–26°C), high riverine output, strong stratification, occasional oxygen depletion and occasional mucous aggregate phenomena (Lipej *et al.*, 2000).

Due to its natural characteristics and the degree of anthropogenic pressure, the area can be considered very sensitive. The Gulf of Trieste, in particular, is subject to substantial chemical, industrial and sewage pollution and is considered one of the most heavily polluted areas in the Mediterranean (Horvat *et al.*, 1999). All the necessary scientific permits for studying dolphins in all parts of the area have been acquired by competent authorities.



**Fig. 1: Locations of dolphin sightings.**  
**Sl. 1: Lokacije opažanj delfinov.**

### Field procedures

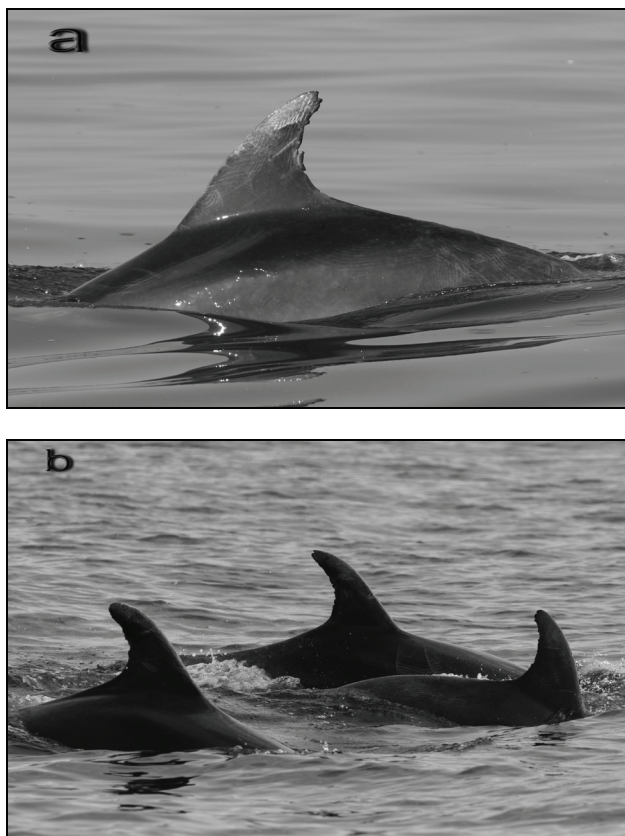
Boat-based and land-based surveys were carried out between July 2002 and September 2008. Two teams often operated simultaneously, one from land and the other from a boat. An attempt was made to keep the search effort of both teams independent. Although surveys were conducted in all seasons, they were mostly concentrated during summer months (July–September), given better general weather conditions and logistic reasons (Tab. 1). Boat-based surveys were carried out using various types of small vessels, mainly rigid inflatable boats with out-board engines. A relatively constant search speed of 25–30 km/h was maintained. An attempt was made to cover all parts of the study area in a given period. However, this was not always possible, given that the effort could vary due to weather conditions, logistic reasons, dolphin

sighting frequency and sighting locations, which could have attracted our attention. Land-based surveys were undertaken from 10–50 m high observation points (mostly cliffs), using binoculars. The first two years of the study (2002–2003) were different from the remaining years both in type (mostly land-based observations were carried out) and the amount of survey effort. From 2004 to 2008, the survey effort was more systematic and involved a greater amount of boat-based effort.

The position of the boat and dolphin groups was determined using a GPS (Global Positioning System) in most cases. At times, when this was not possible due to sightings from land or unavailability of GPS, the position was determined with a compass or using local land marks. The analysis of dolphin distribution was based on the positions obtained at the beginning of each sighting, to avoid the possibility that positions at the end of the

sighting may have been biased by the presence of the research boat. Sightings data were analysed with GIS software ArcView 3.2 and GPS software MapSource 6.13.7.

"Sighting" was defined as an uninterrupted continuous observation of a dolphin focal group. A dolphin focal group was considered any number of dolphins in visual range of the researchers, observed in apparent association, moving in the same direction or staying in the same area and often, but not always, engaged in the same activity. Sightings were subdivided into "sets" (Notarbartolo di Sciara, 1994; Bearzi *et al.*, 1997), in order to account for any change in group size or composition during each sighting. Each set was determined by a change in group size and composition. The mean and median group size and the proportion of groups with offspring were calculated accordingly.

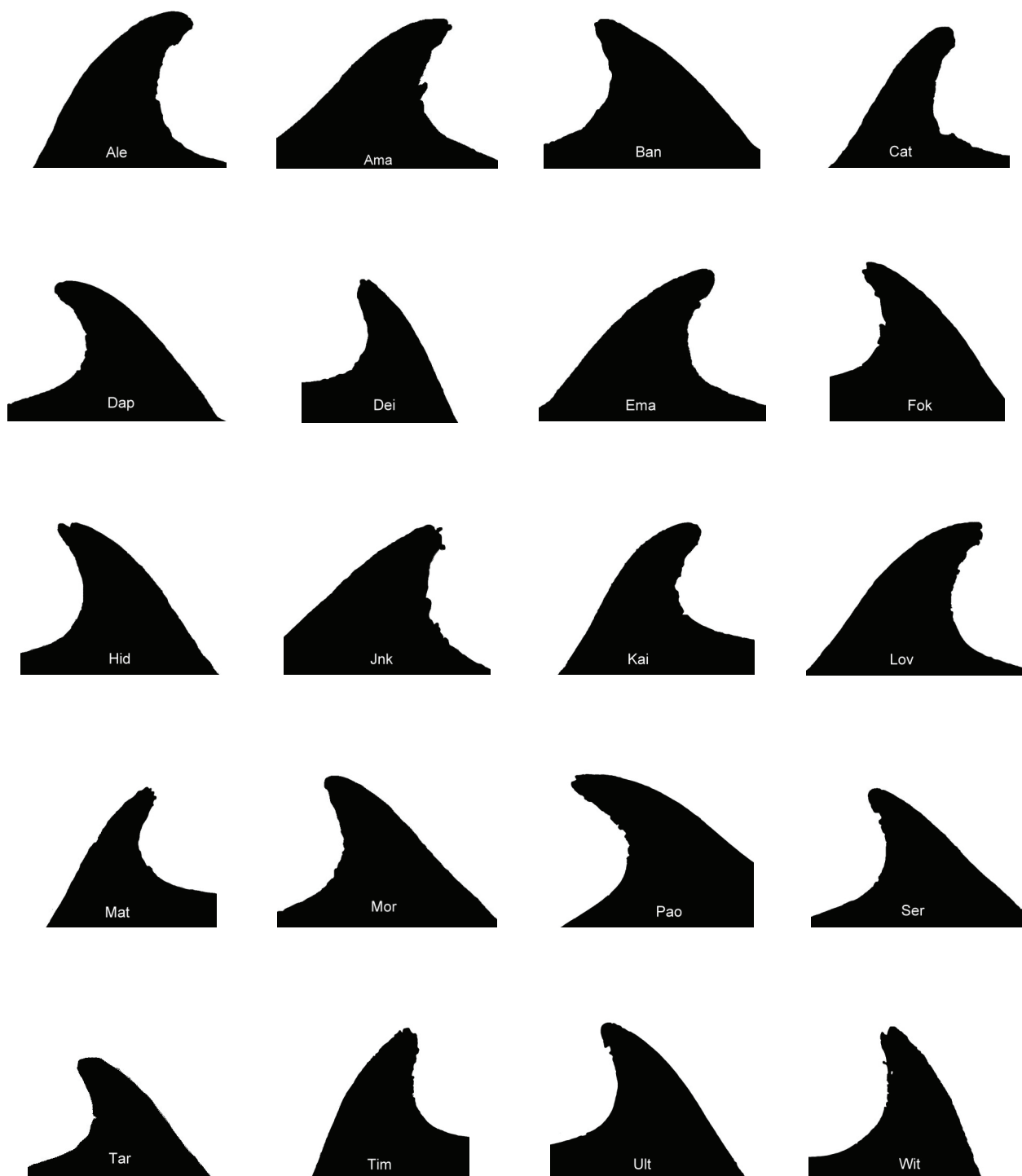


**Fig. 2: Natural marks used for photo-identification. a) Individual Fok, with visible nicks, notches and tooth rakes. b) Individuals Kai, Pao and Lov, showing individual mark differences. (Photo: T. Genov)**  
**Sl. 2: Naravne oznake za foto-identifikacijo. a) Osebek Fok, z vidnimi zarekami, brazgotinami ter sledmi zob. b) Osebki Kai, Pao in Lov kažejo individualne razlike v oznakah. (Foto: T. Genov)**

Survey conditions were considered good if a) the sea state of Beaufort scale was 2 or less; b) at least one experienced observer searched for dolphins (usually 2–5 other observers could participate in the search); c) visibility was not reduced by heavy fog or precipitation. If survey conditions did not match these criteria, no systematic search for dolphins was carried out.

During each survey, navigation and environmental data (time, position, sea state, etc.) were collected every 15 minutes or whenever the direction or conditions of the search changed.

When a dolphin group (or an individual) was found at sea, focal group/individual follow protocol was applied (Mann, 1999, 2000). The group was slowly approached and followed in a way that was believed to cause minimal disturbance to the animals (Wilson, 1995). If a dolphin group was spotted from land, it was either observed from there or subsequently approached with a boat. Dolphins were followed for variable periods of time, usually between 30 minutes and 2 hours, to allow photo-identification of all individuals in the group. Although the time spent following dolphins could vary due to group size and behaviour, an attempt was made to keep it at a minimum to reduce possible disturbance. Standard photo-identification procedures (Würsig & Jefferson, 1990) were carried out during most sightings. Natural marks on dorsal fins, such as nicks, notches, scars, tooth rakes and fin shape were used to identify individual animals (Figs. 2, 3). An attempt was made to photograph both sides of dorsal fins of all members of a dolphin group. Photographs were taken using a SLR camera Nikon F80D equipped with zoom lens Sigma 70–300 mm and ISO 100 or 200 colour transparency films and a digital SLR camera Canon 30D equipped with zoom lens Canon L USM 70–200 mm. More than 10.000 photographs were taken, analysed, labelled and sorted into photo-identification catalogues. New photographs were visually examined and compared to those taken during previous sightings. Two catalogues were compiled: one containing all photographs in chronological order and one containing only the best photographs of each identified individual. To avoid potential bias in the analyses of site fidelity and dolphin abundance, only well-marked animals recognizable from fair and high quality photographs were considered identified. All identified animals were given names as a reference. Poorly-marked or unmarked animals were not considered identified for these analyses. Furthermore, well-marked individuals for which only poor quality photographs were acquired were not considered identified either. These poorly-marked dolphins and those from poor quality photographs were, however, used for group size analysis and kept in the photographic record for possible future re-identifications. Based on capture histories of well-marked animals, we applied mark-recapture models (Otis *et al.*, 1978) for closed popula-



**Fig. 3:** Dorsal fins of 20 photo-identified bottlenose dolphins. These dolphins represent some of the first identified animals in the study area, as well as some of the most well-marked and most resident individuals.

**Sl. 3:** Hrbtne plavuti 20 foto-identificiranih velikih pliskavk. Ti delfini predstavljajo nekatere izmed prvih identificiranih živali na območju raziskave in tudi nekatere izmed najbolj prepoznavnih in stalnih osebkov na tem območju.

tions to estimate the annual abundance of well-marked dolphins frequenting our study area, using the CAPTURE program, run from MARK 4.3 program (<http://www.phidot.org/software/mark/>). In order to build individual capture histories, photo-identification data from sightings were pooled into two different "capture occasion" bouts: one lasting 15 days and the other one month. These two time frames were considered a fair compromise between maintaining a reasonable number of capture occasions per year and the need to allow the necessary remixing of "marked" animals with the rest of the animals using the area. The most appropriate among different annual models was selected using the chi-square test of explained deviance implemented in MARK. Annual estimates of the total number of animals, including offspring, were then calculated by taking into account the estimates of marked animals and the annual proportion of unmarked animals (Wilson *et al.*, 1999). Confidence limits were calculated after Fortuna (2006).

Information on sighting position, time, group size, presence of offspring, behaviour, respiration patterns and interactions with fisheries or maritime traffic was recorded during each sighting. *Ad libitum* behavioural observations (Altmann, 1974; Mann, 1999, 2000) were made throughout the sighting, in order to get an insight into the behaviour of the local population. From 2006 onwards, in addition to *ad libitum* behavioural sampling, a single behavioural sample was taken at the beginning of each sighting, before approaching the focal group (Chilvers *et al.*, 2003). The sample represented the behaviour of  $\geq 50\%$  of the individuals in each group. Groups were scanned to determine behavioural state. This procedure was applied in order to ensure independence of data and avoid pseudoreplication (Chilvers *et al.*, 2003). Behavioural states (travel, dive, dive/travel, active trawler follow, passive trawler follow, socializing, social travel, surface feeding, milling and mixed behaviour) were defined according to objective parameters, following Bearzi *et al.* (1999) and Lusseau (2006).

Group size was assessed in the field and later confirmed through photo-identification. Only sightings where group size could be accurately determined and/or confirmed through photo-identification were used for the analysis of group size.

Offspring were identified according to size, colouration, overall appearance, behaviour and association with adults (for description see Bearzi *et al.*, 1997). Three age classes were used: "adult", "calf" (an offspring more than 1 year old) and "newborn" (an offspring in the first year of its life). Alloparental association ("babysitting") in which offspring associate with individuals other than

their mother (Whitehead, 1996; Mann & Smuts, 1998; Simard & Gowans, 2004), was also recorded.

Gender could opportunistically be determined from photographs of the genital area during aerial behaviour or bowriding (Smolker *et al.*, 1992) or through the identification of mother-offspring pair. Adults consistently and closely accompanied by an offspring were assumed to be females.

Operating trawlers were often opportunistically approached and inspected for possible dolphin presence. All the data such as date and time, position, effort, research platform, number of observers, sea state, dolphin group size, behaviour, presence of offspring, visibility, etc. were recorded onto specifically designed research forms.

Reports of dolphin sightings by fishermen, divers, tourists, local people and other members of the public were collected as an additional source of information. However, such reports were not treated as scientific data and were not included in the analyses, nor merged with the data collected with procedures outlined above, as taking such data into account could lead to significant bias (Zanardelli *et al.*, 1992). If a report of a dolphin sighting resulted in a documented sighting by the research team, however, data from the documented sighting was considered in certain analyses, such as distribution of sighting locations, individual resighting frequencies, group size, presence of offspring, behaviour, interactions with fishery, etc.

## RESULTS

### Distribution, abundance, site fidelity and ranging patterns

Throughout the study period, 258 systematic boat trips, typically lasting 3–6 hours, and 419 systematic land observation sessions, typically lasting 30 minutes to 2 hours, were carried out (Tab. 1). July, August and September were the only months with consistent survey effort. The time spent to find the dolphins ranged from a few minutes to several consecutive days of search. A total of 120 sightings of bottlenose dolphins were recorded (Tab. 1, Fig. 1). Of these, 68 were recorded as a result of boat-based survey effort and 38 as a result of land-based survey effort. The remaining sightings were either a result of opportunistic sightings or successful responses to sighting reports by local people and fishermen. No other cetacean species were encountered during the study.

Tab. 1: Systematic survey effort.

Tab. 1: Sistematično pregledovanje območja.

Year	Month	No. boat surveys	No. land-observation sessions	No. sightings
2002	Jul-Sep	9	0	1
	Oct-Dec	0	0	0
	<b>Year total</b>	<b>9</b>	<b>0</b>	<b>1</b>
2003	Jan-Mar	3	6	8
	Apr-Jun	5	4	5
	Jul-Sep	0	49	8
	Oct-Dec	0	4	0
	<b>Year total</b>	<b>8</b>	<b>63</b>	<b>21</b>
2004	Jan-Mar	0	11	1
	Apr-Jun	1	12	3
	Jul-Sep	20	54	5
	Oct-Dec	2	5	1
	<b>Year total</b>	<b>23</b>	<b>82</b>	<b>10</b>
2005	Jan-Mar	4	2	3
	Apr-Jun	3	12	1
	Jul-Sep	44	54	16
	Oct-Dec	3	0	0
	<b>Year total</b>	<b>54</b>	<b>68</b>	<b>20</b>
2006	Jan-Mar	5	2	1
	Apr-Jun	0	10	0
	Jul-Sep	58	55	26
	Oct-Dec	0	1	0
	<b>Year total</b>	<b>63</b>	<b>68</b>	<b>27</b>
2007	Jan-Mar	0	3	0
	Apr-Jun	5	7	1
	Jul-Sep	41	61	15
	Oct-Dec	0	0	0
	<b>Year total</b>	<b>46</b>	<b>71</b>	<b>16</b>
2008	Jan-Mar	0	0	0
	Apr-Jun	1	0	1
	Jul-Sep	54	67	24
	<b>Year total</b>	<b>55</b>	<b>67</b>	<b>25</b>
<b>TOTAL</b>		<b>258</b>	<b>419</b>	<b>120</b>

Dolphins were seen in the study area in every month of the year except November and December, but survey effort in these two months was very low. The maximum linear distance between two sighting locations of an identified individual was 39 km. Several individuals displayed similar maximum distances between their sighting locations, both within and between years.

A total of 101 well-marked individual dolphins were photo-identified (Figs. 2, 3). Photo-identification data showed that 75% of all dolphins encountered in fully photographed groups were well-marked and could be identified in the long-term. The rate at which new dolphins were identified throughout the study period is shown in figure 4.

The frequency with which identified dolphins were seen in the study area varied greatly (Tab. 2, Fig. 5). Some were seen very often. For example, one adult animal was encountered on 36 occasions (30% of all sightings). Others were observed occasionally and almost half (48%) were seen only once (Fig. 5). Mean frequency of resightings was 5.0 (SD = 7.92,  $n = 101$ , mode = 1, median = 2). The maximum number of times any individual was seen within any given month was 9. A maximum of 27 different individuals were identified in any one month and a maximum of 51 different individuals were identified in any one year. Although there were great differences in the degree of residency among different individuals, some animals displayed a high rate of

**Tab. 2: Residency pattern of 35 selected identified individuals. Black cells represent presence of individuals documented through photo-identification. "Days" represent the total number of days in which photo-identification was carried out in a given month.**

**Tab. 2: Vzorec rezidentnosti 35 izbranih identificiranih osebkov. Črna polja ponazarjajo pojavljanje osebkov v preučevanem območju, "days" (dnevi) pa skupno število dni, v katerih je potekala foto-identifikacija v določenem mesecu.**

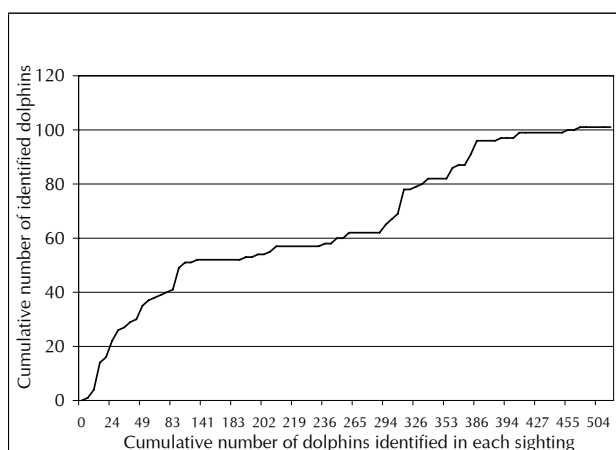
		2003				2004			2005					2006			2007				2008			
ID	Sex	Feb	Mar	May	Jun	Apr	Jul	Aug	Feb	May	Jul	Aug	Sep	Jul	Aug	Sep	May	Jul	Aug	Sep	Jun	Jul	Aug	Sep
<b>Days</b>		2	1	1	1	2	3	2	3	1	9	3	1	9	6	7	1	3	4	1	1	11	4	6
Aar																								
Ale																								
Ama	m																							
Ban																								
Cat	f																							
DaH																								
Dan																								
Dap	f																							
Dei																								
Dor																								
Ema																								
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Gal																								
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Hid																								
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Kai																								
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Lov																								
Mat																								
Mor																								
Nep																								
Pao																								
Ser																								
Sne	f																							
Tar	f																							
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Ves	f																							
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site fidelity and appeared to use the area often and on a yearly basis (Tab. 2). Four individuals were seen in every year from 2003 to 2008, while 8 others were seen in 5 different years (Tab. 2, Fig. 6).

Based on their sighting frequency, individual dolphins could be divided into four arbitrary categories: "common" (20 or more sightings), "frequent" (11–19 sightings), "occasional" (4–10 sightings) and "rare" (fewer than 4 sightings). Individual dolphins could therefore also be divided into three arbitrary categories based on years of resightings: "common" (seen in 5 different years or more), "occasional" (seen in 3 or 4 years) and "rare"

(seen in 2 years or less). The frequency distribution of the number of years in which each individual was seen is shown in figure 6.

In mark-recapture analyses, no significant differences were found between models run on the two different annual datasets: the 15 days vs. one month sampling bout. Results from mark-recapture analyses, based on 15 days datasets, are summarised in Table 3. Estimates from 2003 are not presented, given their extremely high coefficient of variation ( $>0.92$ ). No statistical inter-annual differences were found among estimates ( $p>0.05$ ), except for year 2004, which was different to the remaining years.



**Fig. 4:** Discovery curve showing the number of identified dolphins against the cumulative number of identifiable dolphins encountered during the study.

**Sl. 4:** Krivulja odkrivanja novih delfinov, ki kaže število identificiranih delfinov v primerjavi s kumulativnim številom prepoznavnih delfinov, opaženih v času raziskave.

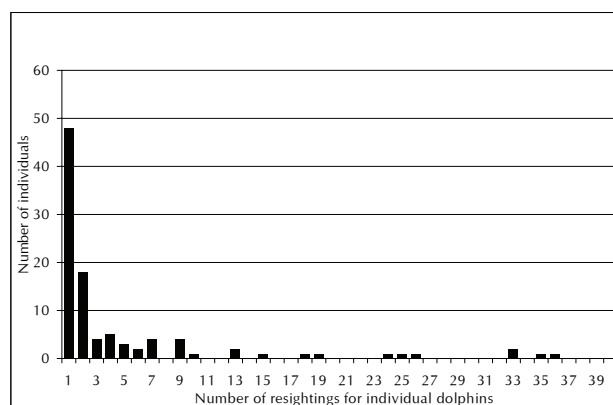
#### Social structure, behaviour and interactions with fishery

The size of dolphins groups ranged from 1 to 43 (Fig. 7). The mean group size calculated from sets was 8 (SD = 7.35,  $n = 90$ , mode = 1, median = 5). Most groups (88.9%) included 15 individuals or less, with a mode of 1 (Fig. 7). Single individuals were represented in 13.3% of the sample, but they did not always seem to represent solitary animals. One adult, for example, was observed following a bottom trawler alone, only to be joined later by 5 other dolphins. Another resident dolphin sighted on two consecutive days in groups of 11 and 13 dolphins, was seen alone the very next day and then sighted in a group of 19 individuals a few days later. On the other hand, one identified adult was only observed once in 2004 and once in 2006. No other dolphins were observed on those days.

Although changes in group size and composition between sightings occurred regularly, with individuals leaving and joining groups, some identified individuals seemed to form relatively stable groups over the study period.

Changes of group size and composition within sightings (a change of set) were observed on only 5 occasions.

Out of 101 photo-identified dolphins, 18 were classified as females, 2 as males and 81 as unknown sex. The most commonly observed groups of identified individuals contained both sexes.



**Fig. 5:** Frequency distribution of the number of times each individual was seen.

**Sl. 5:** Frekvenčna porazdelitev števila opazanj posameznih osebkov.

More than 50 different mother-offspring pairs were observed during the study. Offspring were present in 53.3% of the 105 dolphin groups in which presence or absence of the offspring could be determined. Between 0 and 4 newborns were observed each year: 2 in 2003, 1 in 2004, 1 in 2005, 4 in 2006, 1 in 2007 and 0 in 2008. The year 2002 is excluded due to a small number of sightings. Alloparental associations between offspring and non-mothers were observed on several occasions. Two apparent cases of offspring mortality were observed. Two photo-identified females, which were accompanied by offspring in one year, were seen without one in the next year. Given the size, overall appearance and the estimated age of the offspring (one was a newborn), it is highly unlikely that the offspring had already been weaned. One of the two females was observed with a newborn two years after being sighted without the first calf for the first time.

Some offspring, however, appeared to have survived their first few years. One photo-identified female that was first seen with a newborn in 2004, was still accompanied by a calf in 2006 and 2008. Due to the size and overall appearance of the observed calf, it is believed to be the same individual born in 2004.

Dolphins were observed in all main behavioural states: travel, dive, dive/travel, trawler follow, socializing, social travel, surface feeding and milling.

The behavioural sampling resulted in 61 cases in which behaviour was determined at the beginning of each sighting. Behavioural budget based on this sample is shown in Table 4. Most common behavioural state was "dive-travel" (34.4%), followed by the "active trawler follow" (21.3%) and "travel" (18.1%). Although surface feeding was never recorded at the beginning of the sighting and is therefore not represented in the sample, it was observed during focal group follows in at least 17% of all sightings.

**Tab. 3: Summary of mark-recapture analyses.**

**Legend:**  $N_{\text{hat}}$  = Annual estimate for Well-Marked (WM) animals only; SE = Standard Error;  $N_{\text{TOT}}$  = Annual estimate for marked and unmarked animals;  $D_{\text{TOT}}$  = Annual density for marked and unmarked animals (number of animals / size of the study area in  $\text{km}^2$ ); CV = Coefficient of Variation; 95% CI = Log-normal confidence intervals.

**Tab. 3: Povzetek analiz z metodo "lova in ponovnega ulova".**

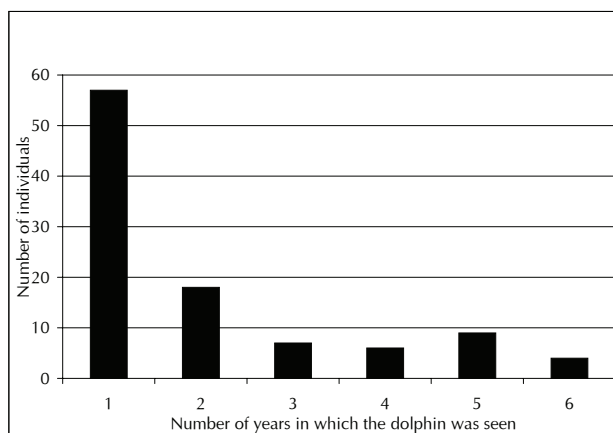
**Legenda:**  $N_{\text{hat}}$  = letna ocena števila označenih živali (WM) živali; SE = standardna napaka;  $N_{\text{TOT}}$  = letna ocena števila označenih in neoznačenih živali;  $D_{\text{TOT}}$  = letna gostota označenih in neoznačenih živali (število živali / velikost raziskovalnega območja v  $\text{km}^2$ ); CV = koeficient variacije; 95% CI = Log-normalni interval zaupanja.

Dataset	Study area ( $\text{km}^2$ )	Model	$N_{\text{hat}}$	SE	Capture occasions	Identified dolphins	Capture probability	%WM	$N_{\text{TOT}}$	$D_{\text{TOT}}$ (n/ $\text{km}^2$ )	CV	95% CI
2003	260	-	-	-	4	22	0.03	0.56	-	-	-	-
2004	550	M(o)	29	3.49	4	24	0.35	0.77	38	0.069	0.17	34-47
2005	1,000	M(th)	41	5.12	6	33	0.23	0.60	68	0.068	0.18	62-81
2006	1,200	M(o)	82	11.97	5	51	0.18	0.76	108	0.090	0.24	84-163
2007	1,400	M(t)	64	13.02	5	36	0.15	0.94	68	0.049	0.36	46-152
2008	1,000	M(t)	42	0.94	7	41	0.37	0.61	69	0.069	0.08	68-70

A relatively high percentage of all dolphin sightings (31.7%) involved an interaction with some type of fishery. In 3.3% of all sightings, interaction was considered likely, but was not confirmed. Known cases of interactions ( $n=39$ ) could further be divided according to gear type: most interactions occurred with pelagic pair trawlers (59%), followed by interactions with bottom trawlers (38.4%). One sighting involved two separate types of interaction (one with pelagic pair trawlers and one with a bottom trawler). Only one case was classified as an interaction with a bottom-set gill net, involving one identified individual.

A group of particular identified individuals resident in the area often followed the same pair of pelagic pair-trawlers. These dolphins often swam rapidly towards the operating trawlers, in order to start following their wake (and presumably to feed). During haul-out of the nets the dolphins sometimes left shortly after, but they often milled in the area, following the trawlers passively. It is not clear whether dolphins also fed on discarded fish. Sometimes the trawlers would move more than 1 km away at normal travel speed and the dolphins would follow. Once the trawlers started trawling again, the dolphins resumed the active follow, which lasted until the next haul-out. At that point, the dolphins milled in the area for a while and then travelled away, usually towards the open sea. The details of how exactly dolphins fed in association with pelagic pair trawlers is yet to be determined.

In contrast, other identified individuals were sighted in the vicinity of the same pelagic pair trawlers, but they did not engage in any type of interactions. Instead, they continued diving in the same area even after the trawlers had passed them.



**Fig. 6: Frequency distribution of the number of years in which each individual was seen.**

**Sl. 6: Frekvenčna porazdelitev števila let, v katerih je bil opažen vsak osebek.**

No incidental mortality in fishing gear (bycatch) was recorded during direct observations of dolphin-fishery interactions; however, one case of bycatch, involving an adult female entangled in a bottom-set gill net, was reported by a local fisherman.

## DISCUSSION

### Distribution, abundance, site fidelity and ranging patterns

Sightings of dolphins seemed to be distributed unevenly across the study area (Fig. 1). Several factors may have contributed to this finding. Firstly, the sightings

distribution was not weighted by effort distribution. Therefore the location of the most suitable and most commonly used land observation point surely affected the distribution of recorded sightings. Furthermore, the starting harbours of boat surveys made areas closer to them more surveyed than those on the outskirts of the study area. Secondly, the study area was continuously expanding from 2003 to 2005, after which it became of a constant size. New sightings were therefore recorded in previously unsurveyed areas. Thirdly, surveys were conducted in a non-systematic way because they were more focused on photo-identification rather than detecting spatial distribution patterns. Furthermore, operating trawlers often attracted our attention. It is therefore clear that a completely equal coverage was not achieved. Nevertheless, some of the most surveyed areas appeared to be used less frequently by the dolphins. Possible bias resulting from differences in dolphin detectability is not likely, because surveys were only carried out in good survey conditions, following the same protocols.

Temporal distribution of survey effort did not allow for comparisons between seasons. There were some differences in distribution between certain years, but that was likely an artefact of the different sampling regime between years, especially in the first few years of the study (land vs. boat surveys, coverage of adjacent areas as the study progressed, etc.).

Dolphin distribution overlapped with areas used intensively by fishermen and many sightings were recorded during interactions with fishery (Genov, 2006a; Genov & Kotnjek, 2007). Dolphins were observed following pelagic pair trawlers actively during trawling and passively during haul-out. Therefore, it is reasonable to assume that fishing operations must have had at least some effect on small-scale movement patterns of dolphins.

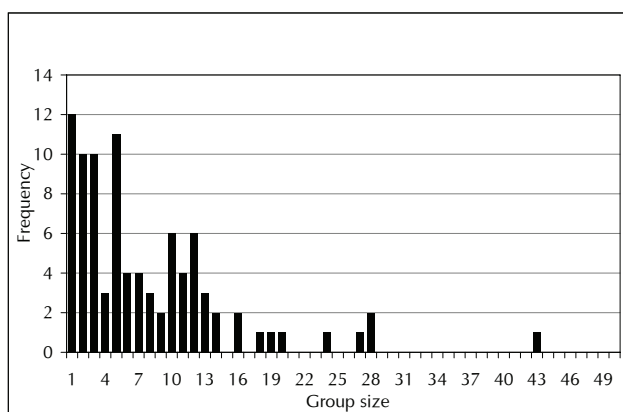
**Tab. 4: Behavioural budget, based on 61 behavioural samples taken at the beginning of each sighting.**

**Tab. 4: Deleži vedenjskih kategorij glede na 61 vedenjskih vzorcev, ki so bili zbrani ob začetku vsakega opazanja.**

Behavioural state	Frequency	%
Dive travel	21	34.4
Active trawler follow	13	21.3
Travel	11	18.1
Dive	5	8.2
Passive trawler follow	2	3.3
Socializing	2	3.3
Social travel	1	1.6
Mixed	6	9.8
<b>Total</b>	<b>61</b>	<b>100</b>

Dolphins often approached the coast to as little as 30 m from shore and entered bays which are used intensively by humans (Fig. 1). It is worth noting that the coastal waters of the study area are used intensively by recreational boats in summer (Morigenos, *unpubl. data*). It has been demonstrated that recreational boating can have strong adverse impacts on dolphins using coastal habitats, affecting their distribution, behaviour and vocalisations and increasing the risk of collisions (Janik & Thompson, 1996; Wells & Scott, 1997; Hastie *et al.*, 2003; Buckstaff, 2004; Fortuna, 2006; Genov, 2006b; Lemon *et al.*, 2006; Lusseau, 2006; Rako, 2006). Preliminary analysis of summer distribution data suggests that dolphins avoid areas close to shore during the day (between 10:00 and 18:00 hrs), heading out to the open sea in the morning when the number of boats increases and approaching the coast again to less than 3 km from shore in late afternoon when the number of boats at sea decreases.

The distances between sighting locations of identified dolphins showed that dolphins are not confined to one small area, but can travel considerable distances in relatively short time, as was demonstrated in other in-shore populations of bottlenose dolphins (Würsig, 1978; Bearzi *et al.*, 1997; Wilson *et al.*, 1997, 2004). The maximum recorded linear distance between two sighting locations of several identified individuals was between 30 and 40 km. Two adults, for example, were photographed in waters off Piran on 8 September 2006 and then again on 11 September 2006 more than 30 km away. However, these distances are likely an artefact of the size of the study area and therefore underestimate the dolphins' true ranging limits. Moreover, great differences were observed in time spent to find the animals. Sometimes dolphins could not be seen on several consecutive surveys, while they could often be found within minutes or hours of search on several other consecutive days. This suggests that dolphin distribution was highly variable and that dolphins ranged within an area much greater than the chosen study area. Dolphins' relatively large ranges, like those recorded in Moray Firth in Scotland (Wilson, 1995; Wilson *et al.*, 1997, 2004), may suggest that the dolphins feed on patchy and unpredictably distributed prey (Wilson, 1995). Additional survey effort in non-summer months is needed in order to acquire insights into distribution patterns in other seasons of the year and to enable comparisons between seasons. Furthermore, this is needed to determine whether dolphin distribution in summer is indeed traffic-related or if it simply reflects dolphins' natural movement patterns.



**Fig. 7: Frequency distribution of group size.**  
**Sl. 7: Frekvenčna porazdelitev velikosti skupin.**

The size of the home range of identified individuals remains unknown; however, a comparison with the photo-identification catalogue of the local population in Kvarnerić, Croatia (Bearzi *et al.*, 1997; Bearzi *et al.*, 1999; Mackelworth *et al.*, 2003; Fortuna, 2006), less than 200 km away, did not yield a single match (Genov & Fortuna, 2005; Genov & Wiemann, 2005).

Resighting rates within and between years have shown a relatively high rate of site fidelity for some individuals (Tab. 2, Fig. 5) although a large number of individuals were sighted only once. In Kvarnerić, only a few animals were sighted once (Bearzi *et al.*, 1997). The discovery curve (Fig. 4) suggests that most dolphins using Slovenian waters on a regular basis have likely been identified. After an initial steep rise (as first dolphins were being identified, resulting in many new dolphins in each photo-identification session), the curve slowly started levelling out, with fewer newly identified animals added to the catalogue. However, given the fact that the study area was probably smaller than the population range, the animals encountered regularly are likely a part of a bigger population. This is supported by the fact that once the curve had started approaching a horizontal asymptote for the first time, it has risen once more in 2006, which corresponds to two sightings of large dolphin groups at the outer edge of the study area, resulting in newly identified dolphins. The previously known dolphins were identified in these two sightings as well. Nevertheless, some recruitment of new animals into the catalogue still occurred even in the original study area alone. For example, two sightings of large groups of dolphins in Piran Bay in summer 2008 resulted in several new identified individuals and therefore a steeper slope of the curve in that period. After this rise, the curve started levelling out again by the end of the study period.

Based on resighting frequencies, 31 dolphins appeared to use the area on a relatively regular basis.

However, the cumulative number of resightings should be interpreted with care, as the number of resightings alone might not necessarily reflect true residency. For example, one particular female and her identifiable calf were seen 9 times in 2005, but were never observed again. In contrast, another individual was "only" observed 7 times: once in 2004, 2005, 2006 and 2007 each and twice in 2008. Another female was seen a few times every second year. All of these individuals were observed in association with highly resident dolphins (those that had the highest resighting rates both within and between years). Is a dolphin sighted several times in a single year more resident than a dolphin that was seen only a few times, but appears to use the area on a yearly basis? For this reason, resighting rates both within and between years should be considered in interpreting these data. In this respect, residency should be regarded as the frequent and long-term use of the area by the animals. When resightings with years as sampling occasions are considered, 26 dolphins appeared to use the area on a relatively regular yearly basis.

Subsequently, those dolphins that matched the categories "common", "frequent" and "occasional" based on their overall resighting frequency and the category "common" and "occasional" based on the number of years in which they were seen were considered resident. This resulted in 25 well-marked dolphins being true residents in the area. This of course does not reflect the total number of animals using the area. Rarely encountered well-marked animals, poorly-marked animals and the lack of survey effort in non-summer months all need to be taken into account.

Mark-recapture estimates (Tab. 3) showed temporal variability of dolphin density and area use, but this could also potentially be due to variation of our spatial and temporal coverage of the study area. Concerning the number of animals frequenting Slovenian territorial waters, the annual density estimates for 2004 and 2008 seem to be good baseline information for conservation management. The annual abundance estimates between these two years are different, but densities are the same. The differences in abundance estimates seem to reflect differences in the size of the study area and the distribution of effort. It should be noted that the amount of effort in 2004 was lower than in 2008, possibly making the estimate for 2008 more reliable. Furthermore, the model  $M_t$  for 2008 allows capture probabilities to vary by time (sampling occasion) and thus possibly making the use of this model more appropriate than the null estimator (model  $M_0$ ) used for 2004 (Wilson *et al.*, 1999). The year 2005 gives good approximation of the number of animals using not only Slovenian territorial waters, but the whole study area. The model  $M_{th}$  used for 2005 allows capture probabilities to vary by time and by individual animal, thus making this model preferable for bottlenose dolphins (Wilson *et al.*, 1999; Bearzi *et al.*, 2008a). The

year 2006 resulted in higher abundance and density estimates, but before any speculation can be made on the possible reasons, more attention should be given to the spatial distribution of the searching effort. As noted above, the estimates for 2006 most likely correspond to two sightings of large dolphin groups at the outer edge of the study area, resulting in higher number of identified dolphins. In 2007, dolphin density was lower compared to other years. This reflects a relatively low number of dolphin sightings in this particular year, despite the wide coverage of the study area. This further suggests variability in dolphin density and the number of animals using the area. The abundance estimate for 2007 is the same as for 2005 and 2008. However, the confidence interval for this year is rather wide, which makes this year inappropriate for baseline information.

In general, even though these estimates should be considered only preliminary, since more thorough evaluation should be given to the annual spatial distribution of effort, all estimates showed good coefficients of variation and a strong consistency among them. The only exception is the estimate for 2003, which is probably biased by the low number of sampling occasions and therefore low number of resightings. Interestingly, annual densities were highly consistent, at least between 2004–2005, despite the strong increase in the size of study area.

#### **Social structure, behaviour and interactions with fishery**

The differences in ecology of different populations and the differences in the definitions of a dolphin group by different authors (these two factors are inherently linked) make it difficult to compare mean group sizes between this and other populations worldwide. Another problem in comparing mean values is in the values themselves. Although most studies provide information on the mean values of group size, these values are poor at describing highly skewed data, as it is often the case with group size data (Wilson, 1995). Median and interquartile range statistics are less influenced by outliers and are therefore better suited to such data (Wilson, 1995).

Both mean and median values of group size from this study, calculated from sets, can however be compared to a study in the same geographical region (the northern Adriatic Sea), using the same methodology and group size definition (Bearzi *et al.*, 1997). The mean group size of 8 is comparable to the mean group size of 7.4 (based on a large sample size of 3-min samples) and the mean group size of 6.75 (obtained by averaging all set sizes, regardless of their duration, which was also a method in the present study) recorded by Bearzi *et al.* (1997) in Kvarnerić, Croatia. This value is also comparable to other populations of inshore bottlenose dolphins, for ex-

ample in Scotland and Florida (Wilson, 1995; Connor *et al.*, 2000). The median value of 5, however, which is often better suited to group size data, was identical to the median value 5 in Kvarnerić (Bearzi *et al.*, 1997) and almost the same as the median value 4.5 in Moray Firth, Scotland (Wilson, 1995). The group size range (1–43) is also very similar to that reported by Wilson (1995) in Moray Firth, Scotland (1–46). However, single animals were not commonly observed in Moray Firth, as they represented 6.3% of the sample (Wilson, 1995), while they represented the modal class and 13.3% of the sample in the present study (Fig. 7).

The size of bottlenose dolphin groups usually increases with the increasing distance from shore (Shane *et al.*, 1986; Connor *et al.*, 2000). Given the characteristics of the northern Adriatic Sea in general and the common use of areas close to shore by the dolphins, this is consistent with relatively small sizes of groups in this study, as they mainly contained less than 15 individuals (Fig. 7). The composition of dolphin groups indicated that, although changes in group size and composition, typical for the fission-fusion societies of bottlenose dolphins (Wilson, 1995; Bearzi *et al.*, 1997; Connor *et al.*, 2000), do occur, some group stability was present. Direct observations of intermixing of dolphin groups within sightings was much less frequent than in Kvarnerić, where a change in group size and composition (a change of set) occurred on average every hour (Bearzi *et al.*, 1997). This could possibly be related to lower dolphin density in the present study area and/or to differences in ecology, social structure and habitat use.

No evidence of sexual or age segregation was found. Frequent observation of mother-offspring pairs (including newborns) and repeated sightings of the same mother-offspring pairs over several years indicate that bottlenose dolphins are breeding and nursing in the area.

No evidence of shark predation was observed. The only species of sharks known to have occasionally fed on bottlenose dolphins in the Adriatic is the great white shark (*Carcharodon carcharias*), which is considered rare in the region at present times (De Maddalena, 2000; Lipej *et al.*, 2004).

Although the sampling techniques and sample size did not allow for any behavioural budget analysis, an useful initial insight was gained into the behaviour of bottlenose dolphins in the study area. Behavioural states dive travel, dive, surface feeding and trawler follow are thought to be linked to foraging or food search (Bearzi *et al.*, 1999). In the present study dive travel, dive, active trawler follow and passive trawler follow (foraging-related behaviours) constitute 67.2% of the total sample size (Tab. 4). Bearzi *et al.* (1999) reported 82% of the behavioural budget to be foraging-related. However, the small sample size in the present study as well as differences in methodology make further comparisons diffi-

cult. Surface feeding, which was not included in the sample, but was observed during at least 17% of the sightings, was reported as rare in Kvarnerić (Bearzi *et al.*, 1999). Dolphin feeding behaviour observed during the study suggests that the study area contains some important habitats for feeding of bottlenose dolphins. Photographs of dolphins tossing mullet (*Mugil* sp.) out of the water during surface feeding were taken. Furthermore, as described above, dolphins often followed pelagic pair trawlers that typically target anchovies (*Engraulis* sp.) and sardines (*Sardina* sp.). This evidence suggests that local bottlenose dolphins regularly feed on mullet, sardines and anchovies, although probably not exclusively. These species are considered a typical part of the diet of bottlenose dolphins in several places in the world, including the Mediterranean Sea (dos Santos & Lacerda, 1987; Barros & Odell, 1990; Blanco *et al.*, 2001; Bearzi *et al.*, 2008b).

A high proportion of sightings involved interactions with fisheries, particularly trawlers, indicating an overlap of target/prey species of fishermen and dolphins. As suggested elsewhere, both fishermen and dolphins are probably drawn to areas of high prey density (Fertl & Leatherwood, 1997). In Kvarnerić, bottlenose dolphins have been estimated to spend around 5% of their time following bottom trawlers (Bearzi *et al.*, 1999), and in some areas of the Mediterranean they have been observed feeding on discarded fish as well (Bearzi *et al.*, 2008b). Such interactions often have negative consequences for at least one party involved. These consequences include dolphin mortality through bycatch, gear damage (either through entanglement of the animals or in the form of holes torn in the net as the dolphins attempt to remove fish), depredation (reduction in the amount or value of the catch as the dolphins mutilate or remove caught fish from the net) and catch loss as the dolphins' presence causes fish to flee from the vicinity of the nets (Reeves *et al.*, 2001; Lauriano *et al.*, 2004). The local fishermen of the pelagic pair trawlers claimed that dolphins caused reduced catches when they followed the trawlers, while gill netters often claimed that dolphins damage their nets and reduce catch. Data collected so far appear to indicate that incidental mortality in fishing gear does not represent a major source of concern for this particular area. However, further systematic studies, possibly based on direct observations onboard fishing boats, should be carried out, as bycatch could also go unreported.

There is also often a positive side to the situation when interactions are concerned, at least for one party. Bottlenose dolphins, which are known for their behavioural adaptability (Shane *et al.*, 1986; Bearzi *et al.*, 2008b), are probably attracted to trawling (and other fishing) activities because they make it easier for the animals to exploit a concentrated food source (Fertl & Leatherwood, 1997). Dolphin distribution might have

been influenced simply by the distribution of their prey, which is also targeted by the fishery, but observations of direct interactions suggest that they were indeed taking advantage of the fishing activities. In contrast, other dolphins seemed less interested in such alternative food sources. It is therefore possible that different groups of dolphins in this population implement different foraging strategies. A study of foraging ecology of Indo-Pacific bottlenose dolphins (*Tursiops aduncus*) by Sargeant *et al.* (2007) showed that various factors such as environmental heterogeneity, demographic and social factors and differences in ecological, genetic and phenotypic differences can shape individual variation in foraging tactics.

The movement patterns and behaviour of at least some dolphin groups appeared to be influenced by fishing activity. Chilvers & Corkeron (2001) and Chilvers *et al.* (2003) found similar results in Moreton Bay, Australia.

## CONCLUSIONS

This study has shown that bottlenose dolphins are a regular, year-round component of the fauna of Slovenian waters, Italian waters of the Gulf of Trieste, and the Croatian waters of north-western Istria. The study has revealed the presence of a resident local population or population segment in these waters, where dolphins were considered rare or occasional visitors. This is the second documented resident local population (or population segment) of bottlenose dolphins in the Adriatic Sea. Moreover, the study has shown that the area likely contains important habitats for bottlenose dolphins inhabiting these waters. These dolphins are part of a larger sub-population of the northern Adriatic Sea, shared by Croatia, Italy and Slovenia. This sub-population therefore needs well-coordinated conservation actions, based on sound science, to ensure its well-being. Bottlenose dolphins under this study have shown that they do not know national borders, as they constantly moved from territorial waters of one country to waters of another. The same is true for human-related threats facing not only dolphins, but the whole northern Adriatic Sea. International collaboration in research, conservation and management of the northern Adriatic ecosystems is therefore fundamental.

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## VELIKA PLISKAVKA (*TURSIOPS TRUNCATUS*) V SLOVENSKIH IN OKOLIŠKIH VODAH SEVERNEGA JADRANA

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### POVZETEK

Med letoma 2002 in 2008 smo avtorji pričujočega prispevka preučevali lokalno populacijo delfinov vrste velika pliskavka (*Tursiops truncatus*) v slovenskih in okoliških vodah (severno Jadransko morje). Raziskovanje je potekalo s plovil in kopenskih opazovalnih točk ter s standardnimi postopki foto-identifikacije. Zabeleženo je bilo 120 opazanj, identificiranih pa 101 osebkov. Stopnja opazanj posameznih delfinov v posameznih letih ter med leti kaže na razmeroma visoko stopnjo pogostosti nekaterih osebkov. Velikost skupin je nihala med 1 in 43. Mladiči so bili zabeleženi v 53,3% skupin. Pogosto so bile opažene matere z mladiči različnih starosti, kar kaže na razmnoževanje vrste in vzrejanje mladičev v preučevanem območju. Zabeleženo vedenje velikih pliskavk je zajemalo vse od dejavnosti, povezanih s hranjenjem, do njihovega potovanja po morju in druženja. Neposredna opazanja njihovih prehranjevalnih dejavnosti dajejo slutiti, da se delfini v tem območju redno hranijo. Opaziti je bilo tudi, da se ribolovna območja ribičev in delfinov pogosto prekrivajo. Sicer pa je letna ocena gostote 0,069 delfina / km<sup>2</sup> verjetno dober osnovni podatek za varstvo in upravljanje velikih pliskavk.

**Ključne besede:** velika pliskavka, *Tursiops truncatus*, ekologija, foto-identifikacija, Slovenija, severni Jadran

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## FUNCTIONAL COMPARISON OF THE SUB-MEDITERRANEAN ILLYRIAN MEADOWS FROM TWO DISTINCTIVE GEOLOGICAL SUBSTRATES

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### ABSTRACT

*In this paper, floristic and functional approaches to the classification of different types of sub-Mediterranean Illyrian grasslands of the association Danthonio-Scorzoneretum villosae (alliance Scorzonetalia villosae, order Scorzonetalia villosae, class Festuco-Brometea) are compared. The data set includes table with 30 relevés from SW Slovenia, sampled in two contrasting geological bedrocks – flysch and limestone – and matrix with 18 traits determined for 119 plant species. We also tested an impact of different geological bedrock on the relative proportions of C-S-R plant strategies in the relevés. With DCA ordination, relevés from limestone and flysch were clearly divided in two groups. First DCA axis suggested a gradient of soil humidity and pH. On the basis of selected traits, 5 Plant Functional Types were clustered and interpreted with Twinspan analysis. PCA ordination of relevés on the basis of plant functional traits revealed that samples from limestone could be separated from those taken on flysch substrate also with functional approach. Relevés from limestone tend to have bigger shares of species, which propagate by seed and vegetatively, and competitors and herbs. Nevertheless, it could be concluded that there are no major functional differences between meadows from both geological substrates. The positions of all relevés in standard C-S-R ternary diagram showed that the relative proportions of C-S-R functional types were not influenced by different geological bedrock.*

**Key words:** dry grasslands, plant functional types, Festuco-Brometea, vegetation, C-S-R plant strategy, SW Slovenia

## CONFRONTO FUNZIONALE DI PRATERIE SUB-MEDITERRANEE ILLIRICHE DI DUE SUBSTRATI GEOLOGICI DISTINTI

### SINTESI

*Nell'articolo vengono confrontati l'approccio floristico e quello funzionale alla classificazione di differenti tipi di vegetazione, sull'esempio di praterie illiriche sub-mediterranee dell'associazione Danthonio-Scorzoneretum villosae (alleanza Scorzonetalia villosae, ordine Scorzonetalia villosae, classe Festuco-Brometea). I dati comprendono una tabella con 30 relevé della Slovenia sud-occidentale, campionati su due substrati geologici contrastanti – flysch e calcare – e una matrice con 18 tratti funzionali determinati da 119 specie vegetali. Gli autori verificano inoltre l'impatto di diversi tipi di substrato geologico sui rapporti relativi delle strategie ecologiche C-S-R delle piante rinvenute nei relevé. Con l'ordinamento DCA, i relevé su calcare e su flysch vengono chiaramente divisi in due gruppi. Il primo asse DCA suggerisce un gradiente di umidità del suolo e del pH. In base ai tratti funzionali selezionati sono stati raggruppati cinque Tipi Funzionali Vegetali ed interpretati con l'analisi Twinspan. L'ordinamento PCA dei relevé, basato sui tratti vegetali funzionali, ha evidenziato che i campioni provenienti dal substrato calcareo potrebbero venir separati da quelli provenienti dal flysch anche con l'approccio funzionale. I relevé del calcare tendono ad avere una porzione più grande di specie, che si riproducono sia vegetativamente che per via sessuata tramite semi, competitori e piante erbacee. Tuttavia gli autori concludono che non ci sono maggiori differenze funzionali tra le praterie di entrambi i substrati geologici. Le posizioni di tutti i relevé nel diagramma ternario standard C-S-R indicano che i rapporti relativi dei tipi funzionali C-S-R non vengono influenzati da substrati geologici differenti.*

**Parole chiave:** praterie secche, tipi vegetali funzionali, Festuco-Brometea, vegetazione, C-S-R strategie vegetali, Slovenia SO

## INTRODUCTION

Classifying plants according to morphology and reproductive attributes has a long history in botany and plant geography (Kleyer, 1999). The renewed interest in classifying species into groups relating to function rather than to taxonomy (e.g. Keddy, 1992; Lavorel *et al.*, 1997; Westoby, 1998; Weiher *et al.*, 1999) has triggered the search for traits that express meaningful differences in ecological behaviour among plant species. There has been an increasing interest in using non-phylogenetic based classifications when the focus is turned on predicting the dynamics of vegetation rather than their taxonomic identity (Gitay, 1999; Cornelissen *et al.*, 2003). Classifying plant species according to their taxonomic and phylogenetic relationships has strong limitations when it comes to answering important ecological questions at the scale of ecosystems, landscapes or biomes (Woodward & Diament, 1991; Keddy, 1992; Körner, 1993). These questions include those on responses of vegetation to atmospheric chemistry, land use and natural disturbance regimes. A promising way for answering such questions (and many other ecological questions) is by classifying plant species on functional grounds (Diaz *et al.*, 2002). These alternative classes are often referred to as plant functional types (PFTs) or groups (Grime *et al.*, 1988; Leishman & Westoby, 1992; Gitay & Noble, 1997).

Plant functional types are non-phylogenetic and non-taxonomic groupings of species and can be defined as groups of plant species sharing similar functioning at the organismic level, similar responses to environmental factors (e.g. temperature, water availability, nutrients, fire and grazing), and/or similar roles in (or effects on) ecosystems or biomes (e.g. productivity, nutrient cycling, flammability and resilience) (Walker, 1992; Chapin *et al.*, 1996; Noble & Gitay, 1996; Diaz & Cabido, 1999; Lavorel *et al.*, 1997; Grime, 2001).

The first step in defining PFTs is to choose a list of key traits that are believed to be important for both understanding and prediction of phenomena relevant for our research. The set of traits or types differ among applications (Woodward & Cramer, 1996).

Plant traits can be obtained by measurements in the field, laboratory, or from the literature. They usually refer to life-history (life span, life cycle), morphology (plant height, lateral spread, life form, spinescence, species leaf area (SLA), and regeneration (e.g. ability to reproduce vegetative, flowering period...) (Kaligarič *et al.*, 2005).

In order to get insights into functional traits, the C-S-R strategy should be considered as well. The C-S-R scheme takes into account a number of different plant traits (Grime *et al.*, 1997; Grime, 2001; Hodgson *et al.*, 1999). The C-S-R plant strategy scheme proposed by Grime (1974) has been widely recognised as a highly

developed plant strategy scheme (e.g. McIntyre *et al.*, 1995; Lavorel *et al.*, 1997; Westoby, 1998). It is built on the assertion that three major determinations of species exist, namely competition (C), stress (S) and disturbance (R). The competitors exploit conditions of low stress and low disturbance, the stress-tolerators are species that occupy habitats with high stress and low disturbance, and the ruderals are adapted to low stress and high disturbance (Grime, 2002).

In this paper we aim to identify different types of sub-Mediterranean Illyrian grasslands of the association *Danthonio-Scorzoneretum villosae* (alliance *Scorzonerion villosae*, order *Scorzoneretalia villosae*, class *Festuco-Brometea*) on the basis of species composition and plant functional traits. The data set includes table with 30 relevés from SW Slovenia, sampled in two contrasting geological bedrocks – flysch and limestone – and matrix with 43 traits determined for 119 plant species.

The first objective was to reveal how the classification of the relevés from limestone and flysch, based on species composition, match with functional classification, based on plant traits. The second objective was to test if different geological bedrock has any impact on C-S-R strategies.

## MATERIALS AND METHODS

## Study area

Study areas are the Primorski kras (Littoral Karst) and Slovenian part of Istria, both within the sub-Mediterranean phytogeographic area (Wraber, 1969) with sub-Mediterranean climate (Ogrin, 1996). Precipitation varies from 900 mm and up to 2,500 mm in the High Karst. The strong "bora" wind causes desiccation and erosion. The mean annual temperature is 12°C to 8°C, minimum is –15°C and maximum 34°C (Kaligarič *et al.*, 2006). The Littoral Karst consists of calcareous limestone, which is penetrable to water, dryer and with more or less alkaline pH reaction. On the other hand, Slovenian Istria is a typical flysch (calcareous sandstone) area. In contrast to calcareous limestone, flysch is a substrate with considerably higher water retention. The soil is humus and moisture rich, pH is neutral or slightly acid (Kaligarič, 1997). Hence, the soil is more fertile.

## Vegetation survey

We analysed 30 vegetation relevés of association *Danthonio-Scorzoneretum villosae*, which were collected in SW Slovenia from both flysch (18 relevés) and limestone (12 relevés) substrates. Relevés were collected using standard procedure of the Braun-Blanquet approach (Braun-Blanquet, 1964; Westhoff & van der Maarel, 1973; Dierschke, 1994).

**Tab. 1: Plant traits, recorded on 119 vascular plant species of sub-Mediterranean Illyrian meadows (SW Slovenia) from two distinct geological substrates, limestone and flysch. Scales of measurement were originally categorical (cat), continuous (cont) or binary (bin).**

**Tab. 1: Rastlinski funkcionalni znaki za 119 rastlinskih vrst submediteranskih ilirskih travnikov (JZ Slovenija) na dveh različnih geoloških podlagah, apnencu in flišu. Podatki so bili v originalu kategorični (cat), zvezni (cont) ali binarni (bin).**

Traits		Abbreviation and description	Data source
Life form	cat	Ch = chamaephytes Ge = geophytes He = hemicryptophytes Th = therophytes	Hegi (1958, 1963, 1964, 1965, 1966, 1974, 1987); Martinčič <i>et al.</i> (2007)
Life cycle	cat	Ann = annual Bien = biennial Peren = perennial	Hegi (1958, 1963, 1964, 1965, 1966, 1974, 1987); Martinčič <i>et al.</i> (2007)
Growth form	cat	Tuss = tussocks Rose = rosette Lea_st = leafy stem Ro_lea = rosette and leafy stem	Hegi (1958, 1963, 1964, 1965, 1966, 1974, 1987); Martinčič <i>et al.</i> (2007); Rothmaler (1995)
Vegetation propagation	cat	VegPro_0 = absent Stol = stolons Rhiz = rhizomes	Hegi (1958, 1963, 1964, 1965, 1966, 1974, 1987); Martinčič <i>et al.</i> (2007); Rothmaler (1995)
Storage organs	cat	Tube = tuber StorOrg_0 = absent	Hegi (1958, 1963, 1964, 1965, 1966, 1974, 1987); Martinčič <i>et al.</i> (2007)
Spinescence	bin	Spin_1 = present Spin_0 = none	Hegi (1958, 1963, 1964, 1965, 1966, 1974, 1987); Martinčič <i>et al.</i> (2007)
Hairiness	cat	HairLow = low HairHigh = high HairNo = no	Hegi (1958, 1963, 1964, 1965, 1966, 1974, 1987); Poldini (1991); Martinčič <i>et al.</i> (2007)
Plant height	cont	Height1 = < 5 cm Height2 = 5-25 cm Height3 = 25-75 cm Height4 = 75-125 cm Height5 = 125-150 cm Height6 = > 150 cm	own measurements
Specific leaf area (SLA)	cont	SLA1 = < 10 mm <sup>2</sup> /mg SLA2 = 10-13 mm <sup>2</sup> /mg SLA3 = 13-16 mm <sup>2</sup> /mg SLA4 = 16-19 mm <sup>2</sup> /mg SLA5 = 19-22 mm <sup>2</sup> /mg SLA6 = > 22 mm <sup>2</sup> /mg	own measurements; LEDA database (Kleyer <i>et al.</i> , 2008)
Leaf dry matter content (LDMC)	cont	LDMC1 = < 5 mg/g LDMC2 = 5-5.29 mg/g LDMC3 = 5.30-5.59 mg/g LDMC4 = 5.60-5.89 mg/g LDMC5 = > 6 mg/g	own measurements; LEDA database (Kleyer <i>et al.</i> , 2008)
C-S-R strategy	cat	C = competitors S = stress-tolerators R = ruderals	own measurements; BiolFlor database (Klotz <i>et al.</i> , 2002)
Flowering start		Flow_St = months	Hegi (1958, 1963, 1964, 1965, 1966, 1974, 1987); Poldini (1991); Martinčič <i>et al.</i> (2007)
Flowering end	cat	Flow_End = months	Hegi (1958, 1963, 1964, 1965, 1966, 1974, 1987); Poldini (1991); Martinčič <i>et al.</i> (2007)
Flowering period	cat	Flow_Per = months	Hegi (1958, 1963, 1964, 1965, 1966, 1974, 1987); Poldini (1991); Martinčič <i>et al.</i> (2007)
Leaf persistence	cat	LP_2 = summer green LP_4 = persistent green	own measurements; BiolFlor database (Klotz <i>et al.</i> , 2002)
Leaf anatomy	cat	Scler = scleromorphic Meso = mesomorphic	own measurements; BiolFlor database (Klotz <i>et al.</i> , 2002)
Type of reproduction	cat	Rep1 = by seed/by spore Rep2 = mostly by seed, rarely vegetatively Rep3 = by seed and vegetatively Rep4 = mostly vegetatively, rarely by seed	own measurements; BiolFlor database (Klotz <i>et al.</i> , 2002)
Guild	cat	Gpoa = grass Gsedge = sedge Gwood = woody plant Gherb = herb Gfab = fabaceae	own measurements; BiolFlor database (Klotz <i>et al.</i> , 2002)

### Selection and measurements of plant traits

While selecting most significant or informative traits, we followed different literature sources (Hodgson *et al.*, 1999; Kahmen *et al.*, 2002; Cornelissen *et al.*, 2003). We selected 18 traits for each species. Traits were chosen from our own database (protocol standardized by Cornelissen *et al.*, 2003). Information on species traits was also taken from two existing trait databases BiolFlor (Klotz *et al.*, 2002) and LEDA (Kleyer *et al.*, 2008). Species was characterized by basic traits as well as composite traits (such as C-S-R strategy). Due to the different species sets, we focused on traits that were easy to measure. The traits selected were: "life form", "life cycle", "growth form", "vegetation propagation", "storage organs", "spinescence", "hairiness", "plant height", "specific leaf area (SLA)", "leaf dry matter content (LDMC)", "CSR strategy", "flowering start", "flowering end", "flowering period", "leaf persistence", "leaf anatomy", "type of reproduction" and "guilds".

The scale of measurement of plant traits was originally continuous or categorical, but they were all transformed into categorical scales for analyses. The list of traits with description of classes in matrix and the sources of information are presented in Table 1.

### Allocating a C-S-R plant functional type to plant species and vegetation samples (relevés)

The C-S-R scheme takes into account a number of different plant traits (canopy height, dry matter content, flowering period, flowering start, lateral spread, leaf dry weight, SLA). To determine the one of 19 C-S-R functional types (Hodgson *et al.*, 1999) for 119 plant species recorded in 30 analysed vegetation relevés of sub-Mediterranean Illyrian meadows (association *Danthonio-Scorzoneretum villosae*), we used data from look-up table with C-S-R types for 1000 European species (source J. G. Hodgson, UCPE Sheffield). For species of unknown type we used a rapid method for attribution of C-S-R type from simple measurements and data published by Hodgson *et al.* (1999).

A functional signature can be derived for a sample of vegetation. The signature is a numerical index which concisely represents the total balance between the different functional attributes that are present among the component species (Hodgson *et al.*, 1999). Using the methodology of Hunt *et al.* (2004), the relative proportions of C-S-R functional types for our 30 samples (relevés) of vegetation were calculated and plotted in C-S-R space.

### Classification and ordination analysis

To classify the relevés according to their species composition, we built a 119 species x 30 relevés matrix

(all matrices available by authors on request). Braun-Blanquet cover-abundance data for the species were converted into a 1 to 9 scale (van der Maarel, 1979). This matrix was then subjected to ordination methods – Detrended Correspondence Analysis (DCA) (Hill & Gauch Jr., 1980). The DCA with detrending by segments was used to estimate the heterogeneity in the species data. Gradient length for the first DCA axis was 3.0, indicating that both the linear and unimodal ordination methods are suitable for the analysis. We decided to use the unimodal (DCA) ordination method (Lepš & Šmilauer, 2003).

In order to identify groups of species with similar traits, we built a 43 traits x 119 species matrix. The scales of measurements of plant attributes were originally continuous, categorical or binary, but they were all transformed into categorical or binary scales prior to the analysis. The number of traits in the matrix increased from 18 to 43 due to the fact that the categorical variables were all re-coded into different numbers of dummy variables – one for each possible level of the factor (Tab. 1). Example: trait "life cycle" has three levels of the factor (1) annual; (2) biennial and (3) perennial species. We submitted the matrix to divisive clustering – Two Way INDicator SPecies ANALysis (TWINSPAN) (Hill, 1979). It was run using the TWINSPAN for Windows version 2.3 (Hill & Šmilauer, 2005).

In order to identify the predominant plant traits for studied meadows, the matrix of 43 traits by 119 species was multiplied by the matrix of 119 species x 30 relevés. The result was a matrix of 43 traits x 30 relevés that was analysed by the means of Principal Component Analysis (PCA) (Goodall, 1954). The values of the traits data were log-transformed prior to PCA analysis.

The ordination methods (PCA, DCA) and visualization of their results were carried out using the Canoco and CanoDraw programs (ter Braak & Šmilauer, 2002).

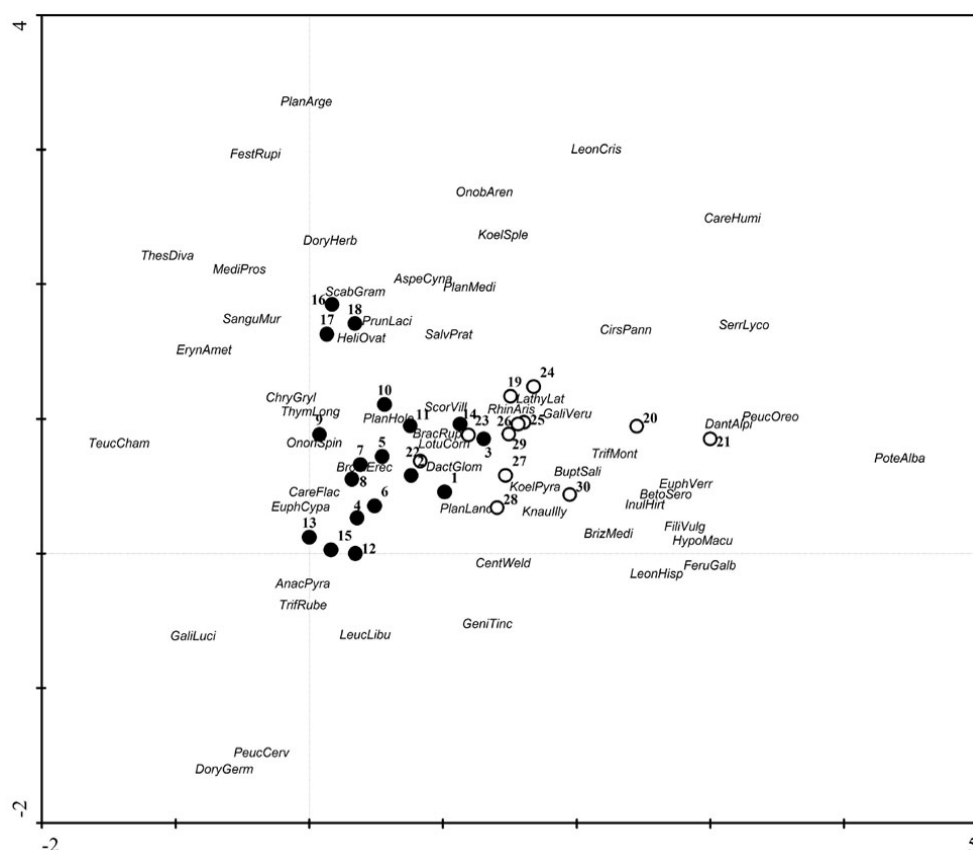
### Nomenclature

Taxonomic nomenclature is in agreement with Martinčič *et al.* (2007), while for the names of the syntaxa we follow Kaligarič (1997).

## RESULTS AND DISCUSSION

### Species composition analysis

The total number of vascular plant species recorded in 30 relevés of studied species rich grasslands was 119 with 88 on limestone (mean =  $30 \pm 5.3$  s. d. per plot, N=12) and 105 (mean =  $34 \pm 4.5$  s.d. per plot, N = 18) on grasslands from flysch. All species are listed in Appendix 1. There are 74 common species, 14 species exclusive to the limestone grasslands and 31 exclusive to the grasslands on the flysch. Higher species diversity of



**Fig. 1:** DCA ordination diagram of relevés ( $N = 30$ , 119 species) of sub-Mediterranean Illyrian meadows from two distinctive geological substrates. Eigenvalues: axis 1 = 0.382; axis 2 = 0.181; 21.3% of variance in species explained by both axes. Shown species have the highest weight. Relevés are divided in two groups according to the geological substrate: ● – relevés from flysch; ○ – relevés from limestone. Abbreviations of species explained in Appendix 1. Relevés numbers correspond to those in Appendix 1.

**Sl. 1:** DCA-ordinacija popisov ( $N = 30$ , 119 vrst) submediteranskih ilirskih travnikov z dveh različnih geoloških podlag. Lastne vrednosti: os 1 = 0,382; os 2 = 0,181; obe osi razložita 21,3% variabilnosti v vrstni sestavi. Prikazane vrste imajo najvišji vpliv. Popisi so ločeni v dve skupini glede na geološko podlago: ● – popisi na flišu; ○ – popisi na apnencu. Razlage okrajšav za vrste so v Prilogi 1. Številke popisov ustrezajo številkam popisov v Prilogi 1.

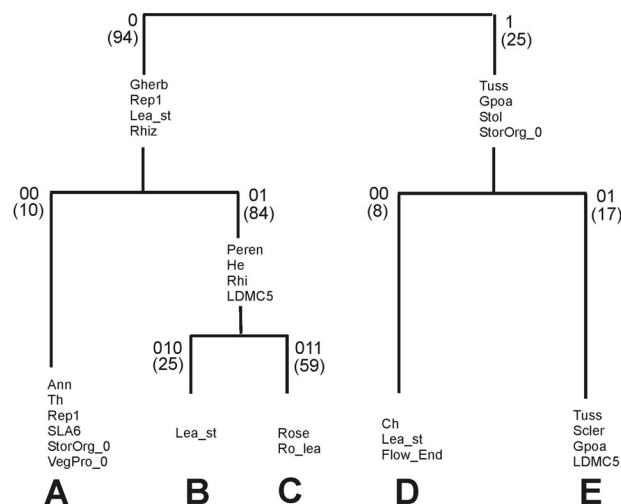
grasslands from flysch is probably connected with higher species pool in this area, which is closer to the Adriatic Sea and milder climate, which contribute a share of Eu-Mediterranean species, lacking on the limestone, being slightly distant from the sea.

To support this statement, we refer to e.g. Poldini (1991): in the atlas of regional flora, one of the richest areas was in lower altitudes, near the sea. Differences in floristic composition were first analysed with DCA analysis of the 119 species  $\times$  30 relevés matrix. DCA ordination is shown in figure 1. Eigenvalues for first two DCA axes are 0.382 and 0.181. First two axes explain together 21.3% variability in the species composition. Relevés are rather continuously arranged along the DCA Axis 1 (Fig. 1). Environmental gradient could be interpreted on the basis of the species ordination. The spe-

cies with the lowest scores (-X) are those characteristically found on deeper soil, with more humus and moisture and neutral to slightly acid pH: *Peucedanum cervaria*, *Dorycnium germanicum*, *Teucrium chamaedrys*, *Galium lucidum*, *Trifolium rubens*, *Chrysopogon gryllus* and *Carex flacca*. *Potentilla alba*, *Hypochoeris maculata*, *Carex humilis*, *Inula hirta* were some of the species receiving the highest scores in the first DCA, i.e. they are associated with sites with more warm, dry and basiphilous conditions. Dispersion of relevés along the first axis of the DCA suggested a gradient of soil humidity and soil pH. Relevés of the most humid and neutral to slightly acid soil on the flysch (Nos. 1 to 18) are positioned on the left side, and relevés of the very dry shallow limestone soils with high pH (No. 19–30) are positioned on the right side of the DCA ordination biplot.

### Plant functional types

On the basis of Twinspan analysis of the 43 traits x 119 species matrix (Fig. 2), we distinguished five groups (clusters) that could be interpreted as plant functional types (PFT).

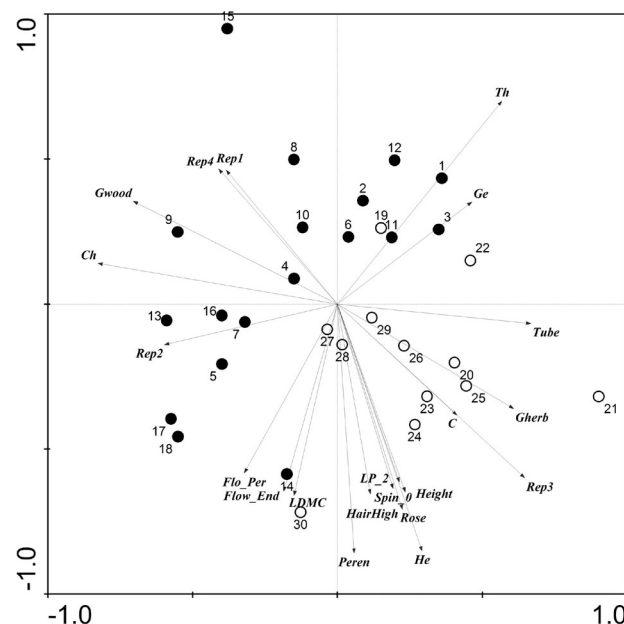


**Fig. 2: Simplified TWINSpan classification tree (dendrogram) of 119 species. For each division number of group, numbers of species (in brackets) in group and indicator traits are shown. Abbreviations of plant traits are explained in Table 1.**

**Sl. 2: Poenostavljen diagram TWINSpan-klasifikacije (dendrogram) za 119 vrst. Za vsako delitev so prikazani število skupine, število vrst v skupini (v oklepaju) in indikatorski funkcionalni znaki. Okrajšave za funkcionalne znake so razložene v Tabeli 1.**

Group A included 10 species. They were all annual species (therophytes) (Ann, Th) with relatively high values for SLA (SLA6). Height values of SLA tend to correspond with relatively low investments in leaf "defences" and short leaf lifespan. Species in resource-rich environments tend to have larger SLA than those in environments with resource stress (Cornelissen *et al.*, 2003). Species from group A reproduce by seeds (Rep1), they do not have storage organs (StorOrg\_0) and they do not propagate vegetatively (VegPro\_0). We could find here *Blackstonia perfoliata*, *Linum catharticum*, *Rhinanthus* spp., *Trifolium campestre* and *Centaurea erythraea*. Groups B and C represented perennial species (Peren), hemicryptophytes (He) with rhizomes (Rhi) and with higher values for LDMC (LDMC 5). Species from group B were mostly tall hemicryptophytes (He) with leafy stem (Lea\_st), like *Achillea collina*, *Bupththalmum salicifolium*, *Centaurea triumfetti* subsp. *adscendens* and *C. pannonica*, *Leucanthemum liburnicum*, *Dianthus tergestinus* and *Onobrychis arenaria*. Group C is characterized by rosette (Rose)

and hemirosette (Ro\_lea) plants: *Carlina acaulis*, *Eryngium amethystinum*, *Globularia punctata*, *Hypochoeris maculata*, *Leontodon hispidus*, *Plantago* spp., *Potentilla alba*, *Tragopogon orientalis* and *Trifolium montanum*. The last two groups, D and E, were separated from others already at the first cut level. 8 species from group D were mainly chamaephytes (Cham) with leafy stem: *Chamaespartium sagittale*, *Teucrium chamaedrys* and *Thymus longicaulis*. In comparison to others, this group included significantly higher share of late flowering species. The most homogenous was group E with 17 species, where we could find tussock-forming species, with scleromorphic leaves and high LDMC values. All this information indicated that this group was rich on grasses (fam.



**Fig. 3: PCA ordination diagram of matrix 3 with 30 relevés and 43 traits. Eigenvalues: axis 1 = 0.30; axis 2 = 0.18; 42.6% of variance in species explained by both axes. Shown traits (21) have the highest weight. Relevés divided in two groups according to the geological bedrock: ● – relevés from flysch; ○ – relevés from limestone. Abbreviations of plant traits explained in Table 1. Relevés numbers correspond to those in the Appendix 1.**

**Sl. 3: PCA-ordinacija matrike 3 s 30 popisi in 43 rastlinskimi funkcionalnimi znaki. Lastne vrednosti: os 1 = 0,30; os 2 = 0,18; obe osi razložita 42,6% variabilnosti v vrstni sestavi. Prikazani funkcionalni znaki (21) imajo najvišji vpliv. Popisi ločeni v dve skupini glede na geološko podlago: ● – popisi na flišu; ○ – popisi na apnencu. Razlage okrajšav za funkcionalne znake so v Prilogi 1. Številke popisov ustrezajo številkam popisov v Prilogi 1.**

Poaceae) and grass-like plants (fam. Cyperaceae) (e.g. *Anthoxanthum odoratum*, *Bothriochloa ischaemum*, *Brachypodium rupestre*, *Briza media*, *Bromopsis erecta*, *Carex flacca* and *C. humilis*, *Chrysopogon gryllus*, *Danthonia alpina* and *D. decumbens*, *Festuca pratensis* and *F. rubra*, *Koeleria pyramidata*, *Poa pratensis*...).

A PCA ordination of matrix 3 with 43 traits x 30 relevés was performed in order to differentiate the analyzed sub-Mediterranean Illyrian meadows on the basis of five PFT according to the two distinct geological substrates. Ordination graph is presented in figure 3. Only 21 traits with the highest weight (most significant) are shown in the ordination diagram. The angles between arrows indicate correlations between traits. Relevés with high proportion of species reproducing by seeds and vegetatively (Rep3) had also high shares of herbaceous plants (Gherb) plants and competitors (C). Relevés with high proportion of hemicryptophytes (He) had also many perennial (Peren) and tall species (Height).

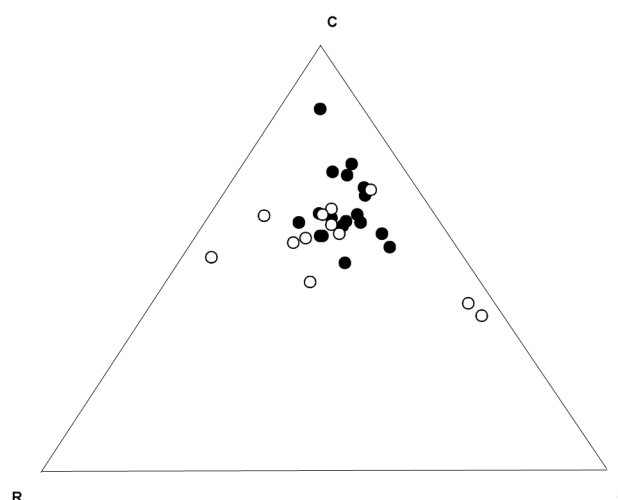
Traits the most correlated with relevés scores of PCA axis 1 (eigenvalue = 0.30) were tuber (Tube) and chamaephytes (Ch).

In the PCA ordination of all relevés on the basis of plant traits (Fig. 3), relevés from limestone were grouped in the lower right side of the ordination biplot. Those relevés had bigger shares of species, which propagate by seed and vegetatively (Rep3), competitors (C) and herbs (Gherb). Nevertheless, it could be concluded that there are no major functional differences between meadows from both geological substrates.

### CSR strategies

In figure 4, the positions of all calculated signatures for 30 relevés of sub-Mediterranean Illyrian meadows from two contrasting geological substrates are presented in standard C-S-R ternary diagram. Relevés are grouped in the upper part of the triangle, showing relative im-

portance of C component (competition). The second objective was to test if different geological bedrock resulted in significant differences in C-S-R strategies. No significant differences was found, except for the two relevés from limestone: they show influence of stress-tolerators (wind-exposed positions, dryer calcareous substrate), and a few relevés from flysch have higher impact of C component (consisted of typical competitors in fertile soil and favourable climate). Unless it could be concluded that the relative proportions of C-S-R functional types are not influenced by different geological bedrock.



**Fig. 4: C-S-R ordination of vegetation of relevés (N = 30, 119 species) of sub-Mediterranean Illyrian meadows from two contrasting geological substrates. ● – relevés from flysch; ○ – relevés from limestone.**

**Sl. 4: C-S-R-ordinacija popisov (N = 30, 119 vrst) sub-mediterranskih ilirskih travnikov iz dveh različnih geoloških podlag. ● – popisi na flišu; ○ – popisi na apnencu.**

## FUNKCIONALNA PRIMERJAVA SUBMEDITERANSKIH ILIRSKIH TRAVNIKOV Z DVEH RAZLIČNIH GEOLOŠKIH PODLAG

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### POVZETEK

V pričujočem članku smo primerjali floristični in funkcionalni pristop pri klasifikaciji tipov vegetacije na primeru sub-mediterranskih ilirskih travniških asociacij *Danthonio-Scorzoneretum villosae* (zveze *Scorzonerion villosae*, reda *Scorzoneretalia villosae*, razreda *Festuco-Brometalia*). Baza podatkov je obsegala tabelo s 30 popisi travnišč iz SZ Slovenije, zbranih na dveh različnih geoloških podlagah – flišu in apnencu – in matrike z 18 funkcionalnimi znaki, ki smo jih določili za 119 rastlinskih vrst. Naš cilj je bil tudi ugotoviti, ali različna geološka podlaga vpliva na deleže posameznih C-S-R-ekoloških strategij rastlin v popisih.

Najprej smo analizirali raznolikost floristične sestave z indirektno analizo gradientov (DCA) in na podlagi ordinacije popisov in vrst ugotovili, da so se popisi razporedili sorazmerno zvezno vzdolž gradienta vlažnosti (oz. sušnosti) ter kemijske reakcije (pH) tal. Popisi z različnih geoloških podlag so bili med sabo jasno ločeni. S klasifi-kacijsko analizo Twinspan matrike 43 funkcionalnih znakov  $\times$  119 rastlinskih vrst smo določili 5 skupin vrst, ki smo jih interpretirali kot funkcionalni tipi, ki se pojavljajo na obravnavanih suhih traviščih.

Za ugotavljanje razlike med popisi na apnencu in flišu na podlagi izbranih funkcionalnih znakov rastlin smo matriko 43 funkcionalnih znakov  $\times$  119 vrst pomnožili z matriko 119 vrst  $\times$  30 popisov. Kot rezultat smo dobili matriko 43 funkcionalnih znakov  $\times$  30 popisov, ki smo jo nato analizirali z indirektno ordinacijsko metodo glavnih komponent (analizo PCA). Na grafu se je na podlagi funkcionalnih znakov rastlin oblikovalo kar nekaj manjših skupin popisov. Tudi tokrat so se popisi iz apnenca razporedili skupaj, in sicer desno spodaj v grafu. To pomeni, da so med zbranimi funkcionalnimi znaki tudi takšni, ki imajo večje deleže vrst (npr. kompetitorji (C), zelišča (Gherb), razmnoževanje s semeni in vegetativno (Rep3)) ali pa manjše deleže vrst (npr. lesne vrste (Gwood)) v popisih na apnencu v primerjavi s popisi na flišu. Vendar pa po analizi teh znakov ugotavljamo, da le-ti nimajo večjega pomena in zaključujemo, da ni značilnih razlik med travišči na apnencu in flišu glede na funkcionalne znake in tipe rastlin. Tudi razporeditev popisov v trikotniku C-S-R na podlagi relativnih deležev posameznih C-S-R-ekoloških strategij rastlin v popisih ne dokazuje značilnih razlik med popisi na apnencu in flišu.

**Ključne besede:** suhi travniki, rastlinski funkcionalni tipi, *Festuco-Brometea*, vegetacija, C-S-R strategije rastlin, JZ Slovenija

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**Appendix 1: 30 relevés of sub-Mediterranean Illyrian grasslands of the association *Danthonio-Scorzoneretum villosae* (alliance *Scorzonerion villosae*, order *Scorzoneretalia villosa*, class *Festuco-Brometea*). Relevés were sampled in SW Slovenia in two contrasting geological bedrocks – flysch and limestone.**

**Priloga 1: 30 popisov submediteranskih ilirskih travnikov asociacije *Danthonio-Scorzoneretum villosae* (zveza *Scorzonerion villosae*, red *Scorzoneretalia villosae*, razred *Festuco-Brometea*). Popisi so bili zbrani v JZ Sloveniji na dveh različnih geoloških podlagah – fliša in apnenca.**

Species	Relevé number	Flysch																	Limestone												
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Species abbreviations	Number of species per relevé	29	44	32	31	38	35	37	35	32	29	28	31	28	37	30	40	35	36	27	26	26	24	28	28	24	29	41	38	30	33
<i>Achillea collina</i>	<i>AchiColl</i>									+							+	+	+								+				
<i>Anacamptis pyramidalis</i>	<i>AnacPyr</i>		+	+		+	+		+		+	+	+																		
<i>Anthericum ramosum</i>	<i>AnthRamo</i>														+						+	1									
<i>Anthoxanthum odoratum</i>	<i>AnthOdor</i>																											1	1	1	
<i>Anthyllis vulneraria</i> subsp. <i>polyphylla</i>	<i>AnthPolyp</i>			+		+										+				+					+						
<i>Asperula cynanchica</i>	<i>AspeCynan</i>												+		+	+	+	+	1	+				+		+	+	+	+		
<i>Asperula purpurea</i>	<i>AspePurp</i>							1							+		+														
<i>Betonica serotina</i>	<i>BetoSero</i>	2	+	+											+	+				+	+				+	+	1				1
<i>Blackstonia perfoliata</i>	<i>BlacPerf</i>				r		+	+	+							+															
<i>Botryochloa ischaemum</i>	<i>Botrlsch</i>																	1	1	1											
<i>Brachypodium rupestre</i>	<i>BracRupe</i>	+	1	1	1	2	3	1	1		1	1	3	+	2		2	1	2		1			1		1	+	2	1		2
<i>Briza media</i>	<i>BrizMedi</i>	1	2	2	+	1	1	1	1	+		1		+	+	+				1	1	2	1	2	+	1	2	1	1	1	1
<i>Bromopsis erecta</i>	<i>BromErec</i>	2	3	4	2	2	4	2	3	2	2	2	3	2	4	3	3	3		1		2	2		1	3	2	2	3	1	
<i>Buphthalmum salicifolium</i>	<i>BuptSalic</i>		+	+	+	1	1					1	+		+	+		+		+	+	+		+	+	1	+	+		+	
<i>Campanula glomerata</i>	<i>CampGlom</i>																				+	+									
<i>Campanula rapunculus</i>	<i>CampRapu</i>										+	+					+											+	+	+	
<i>Carex flacca</i>	<i>CareFlac</i>	+	+		+	2	1	+	+	+	+	+	+	+	+	+		1		1		+				2					
<i>Carex humilis</i>	<i>CareHumi</i>														1		1	1		2	2	2		+							
<i>Carex montana</i>	<i>CareMont</i>																											1	1	1	
<i>Carlina acaulis</i>	<i>CarlAcau</i>		+																		+										
<i>Carlina corymbosa</i>	<i>CarlCory</i>					+								+		+	+	+													
<i>Centaurea triumfettii</i> subsp. <i>adscendens</i>	<i>CentAdsc</i>																+	+												+	
<i>Centaurea pannonica</i>	<i>CentWeld</i>	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+			+	+	+	+	+	+	+	2	1	+	1	
<i>Centaureum erythraea</i>	<i>CentEryt</i>					+						+			+		+														
<i>Chamaespartium sagittale</i>	<i>ChamSagi</i>					+																									
<i>Chrysopogon gryllus</i>	<i>ChryGryl</i>	3	2	2	4	2		1	3	3	4	3	+	1	1	2	3	3	2				3				2	2			
<i>Cirsium pannonicum</i>	<i>CirsPann</i>		+	1				+				+								1			1	1		3	+		+	1	
<i>Dactylis glomerata</i>	<i>DactGlom</i>		1	+	1	+		1	1		+	+	1	+	+	+	1	1	1				+		1	1	+	1	3		
<i>Danthonia alpina</i>	<i>DantAlpi</i>	+	2	3																2	3		+	+	+		1	1			
<i>Danthonia decumbens</i>	<i>DantDecu</i>																			1	1										
<i>Daucus carota</i>	<i>DaucCaro</i>				+												+	+	+									1			
<i>Dianthus tergestinus</i>	<i>DianTerg</i>															+		+	+												
<i>Dorycnium germanicum</i>	<i>DoryGerm</i>				2	1	1	+				1	2		+								1					+			
<i>Dorycnium herbaceum</i>	<i>DoryHerb</i>	1	1	2					+		2						1	1													
<i>Eryngium amethystinum</i>	<i>ErynAmet</i>	r	1			+	+	+	1	+					+	+	+	1	1	+											
<i>Euphorbia cyparissias</i>	<i>EuphCypa</i>		+			+							+	+	+		1						+								

	Relevé number Number of species per relevé	Flysch																	Limestone												
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
		29	44	32	31	38	35	37	35	32	29	28	31	28	37	30	40	35	36	27	26	26	24	28	28	24	29	41	38	30	33
Species	Species abbreviations																														
<i>Euphorbia nicaeensis</i>	<i>EuphNica</i>				1	1		+	+				+		+																
<i>Euphorbia verrucosa</i>	<i>EuphVerr</i>	1	+	1				+													1		+		+	+	+	1	1		
<i>Ferulago galbanifera</i>	<i>FeruGalb</i>			+			+				r		1							1	1		+	+	+		1		2		
<i>Festuca rubra</i>	<i>FestRubr</i>																											1	2		1
<i>Festuca rupicola</i>	<i>FestRupi</i>								+	+			+			2	2	2		+	+										
<i>Filipendula vulgaris</i>	<i>FiliVulg</i>	+		+							+			1						+	1	+	+		+	+	2	1		2	
<i>Fumana procumbens</i>	<i>FumaProc</i>															r	+														
<i>Galium lucidum</i>	<i>GaliLuci</i>	1	+	+		+	+	1					+	2		1	+	1													
<i>Galium verum</i>	<i>GaliVeru</i>				2				+	+	2	2		2						+		+	+	+		+	1	1	1	2	
<i>Genista germanica</i>	<i>GeniGerm</i>							r								+											+	1		+	
<i>Genista tinctoria</i>	<i>GeniTinc</i>				+	+		+						+	+												+	1		+	
<i>Geranium sanguineum</i>	<i>GeraSang</i>						+							1																	
<i>Gladiolus illyricus</i>	<i>GladIlly</i>	+																			+										
<i>Globularia punctata</i>	<i>GlobPunc</i>		+						+					+	+																
<i>Gymnadenia conopsea</i>	<i>GymnCono</i>					+	+														+										
<i>Helianthemum ovatum</i>	<i>HeliOvat</i>		1			+	+		+	+	+	+			1	1	1	+		+							+	1	+		+
<i>Hieracium pilosella</i>	<i>HierPilo</i>							+		+				+	1		+														
<i>Hippocrepis comosa</i>	<i>HippComo</i>													+	1		+											+			
<i>Hypericum perforatum</i>	<i>HypePerf</i>							+																					+		
<i>Hypochoeris maculata</i>	<i>HypoMacu</i>																			+	+			+	+	1	2	3		1	
<i>Inula hirta</i>	<i>InulHirt</i>	2	1	2	+	+														+	+	+	+		+	+					
<i>Knautia illyrica</i>	<i>KnaulIly</i>	+	2		+	+	+	+	+	+	+	+	+	+				+		+	+	+	+	+	+	+	1	1	1	+	
<i>Koeleria pyramidata</i>	<i>KoelPyra</i>	+	+	1				2	+		+	+		+									+	+	+	+	2			2	
<i>Koeleria splendens</i>	<i>KoelSple</i>				+	2	+									1		1		+	+										
<i>Lathyrus latifolius</i>	<i>LathyLaty</i>			+				r	+	+	+	+	+											1	+	+		+	+	1	+
<i>Lathyrus pratensis</i>	<i>LathPrat</i>					+		+					+					+										+	1		
<i>Lembotropis nigricans</i>	<i>LembNigr</i>				+								+	+	+	+	+														
<i>Leontodon crispus</i>	<i>LeonCris</i>															+	+	+	+	+	+			+					1		
<i>Leontodon hispidus</i>	<i>LeonHisp</i>						+					1								1		+	1	+			1	1	1	1	
<i>Leucanthemum liburnicum</i>	<i>LeucLibu</i>	+	+	+		+	r		2	+				+									+	+			+	+			
<i>Linum catarthicum</i>	<i>LunCath</i>	+	+	+																+		+					+		+		
<i>Linum flavum</i>	<i>LinuFlav</i>		+	2																											
<i>Linum tenuifolium</i>	<i>LinuTenu</i>	+	+			+								+		+	+	+	+												
<i>Lotus corniculatus</i> subsp. <i>hirsutus</i>	<i>LotuCorn</i>	+		+	+	+	+	1	+	+	1	+	+	+	+	+	+	+	+	+		1		+		+	+	+	+	1	+
<i>Medicago falcata</i>	<i>MediFalc</i>					+							+	+			+														
<i>Medicago prostrata</i>	<i>MediProst</i>		+		+	+		1	1	1	1	2				+	+	+	+			+	+								
<i>Odontites lutea</i>	<i>OdonLute</i>		+										+			+															
<i>Onobrychis arenaria</i>	<i>OnobAren</i>		1													1	+	1		1				+	1	+	+			2	
<i>Ononis spinosa</i>	<i>OnonSpin</i>	+	+		1	2	1	2	+	+	2	1	+	+		1		+				+	+		+			1	1		
<i>Pastinaca sativa</i>	<i>PastSati</i>											r		+																	
<i>Peucedanum cervaria</i>	<i>PeucCerv</i>	2	3		+		+	2	+			2			2																
<i>Peucedanum oreoselinum</i>	<i>PeucOreo</i>													+						1	1		+					+	3		
<i>Picris hieracioides</i>	<i>PicrHier</i>								+			+				+	+	+													
<i>Pimpinella saxifraga</i>	<i>PimpSaxi</i>	1												+																	

	Relevé number Number of species per relevé	Flysch																Limestone														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	
		29	44	32	31	38	35	37	35	32	29	28	31	28	37	30	40	35	36	27	26	26	24	28	28	24	29	41	38	30	33	
Species	Species abbreviations																															
<i>Plantago argentea</i>	<i>PlanArge</i>															2	2					+		+		+						
<i>Plantago holosteum</i>	<i>PlanHolo</i>		+		+	+		1	+	+								+		+			+	+	+				+			
<i>Plantago lanceolata</i>	<i>PlanLanc</i>				+		+			1			+		+			+	+									2	1	1	1	
<i>Plantago media</i>	<i>PlanMedi</i>		+	1		+	1	1			1	1		+	+		1	1	+		+			1	2	1	+	1	1	+		
<i>Poa pratensis</i>	<i>PoaPrat</i>																												+		+	
<i>Polygala nicaeensis</i> subsp. <i>mediterranea</i>	<i>PolyNicae</i>																						1									
<i>Potentilla alba</i>	<i>PoteAlba</i>																			2	3										1	
<i>Potentilla australis</i>	<i>PoteAust</i>															+									+		+					
<i>Potentilla erecta</i>	<i>PoteErec</i>																					+							+	+		
<i>Prunella grandiflora</i>	<i>PrunGrand</i>					+														+	+				+	+						
<i>Prunella laciniata</i>	<i>PrunLaci</i>		+		+				+	1		+						+							+							
<i>Ranunculus bulbosus</i>	<i>RanuBulb</i>																+		+													
<i>Ranunculus nemorosus</i>	<i>RanuNemo</i>																											+	+		1	
<i>Rhinanthus glacialis</i>	<i>RhinAris</i>		+	+									+			+	1			+	+	+	+	+	1	1	+					
<i>Rhinanthus freynii</i>	<i>RhinFrey</i>				+				+																				+			
<i>Rhinanthus minor</i>	<i>RhinMino</i>		+								2																	+	+	+		
<i>Rumex acetosa</i>	<i>RumeAcet</i>																											+		+		
<i>Salvia pratensis</i> agg.	<i>SalvPrat</i>			+		+	+	+	+	+	1	2	+			+	+			+			1		+	+	+	+	+	+		
<i>Sanguisorba muricata</i>	<i>SanguMuri</i>		+		+	+	+	+	+	+	+	+					+	+					+	+								
<i>Satureja montana</i> subsp. <i>variegata</i>	<i>SatuVari</i>					+												+														
<i>Scabiosa grammuntia</i>	<i>ScabGram</i>			+			+	+			1	1				+	+	+					+	+			+					
<i>Scorpiorus subvillosus</i>	<i>ScorSubv</i>									+	+	+																				
<i>Scorzonera villosa</i>	<i>ScorVillo</i>		1			1		2	2	2	2	+	+	2		+	+	2		3	+			2	3	2	+	1	1	2	+	
<i>Senecio jacobaea</i>	<i>SeneJaco</i>		r												+													+	+			
<i>Serratula lycopifolia</i>	<i>SerrLyco</i>																					1	1	1		+	2	1				
<i>Stachys recta</i> agg.	<i>StacRect</i>						+	+					+	+																		
<i>Teucrium chamaedrys</i>	<i>TeucCham</i>		1						+	+			+	+		+	1															
<i>Teucrium montanum</i>	<i>TeucMont</i>		+	1			+		+					+						+												
<i>Thalictrum minus</i>	<i>ThalMinu</i>			+											+					+	+										+	
<i>Thesium divaricatum</i>	<i>ThesDiva</i>		+		+		+	2		1						1	+	+														
<i>Thymus longicaulis</i>	<i>ThymLong</i>		+	+		+	+		+	+	+	+		+		+	+	1	+		+							+				
<i>Tragopogon pratensis</i> subsp. <i>orientalis</i>	<i>TragOrie</i>		+	+						+	+			+						+							+					
<i>Tragopogon tommasinii</i>	<i>TragTomm</i>				+	+	1	+																								
<i>Trifolium campestre</i>	<i>TrifCamp</i>								1	+		+																				
<i>Trifolium montanum</i>	<i>TrifMont</i>					+	2				+				+					+	+		+	+		+	1	2	2			
<i>Trifolium pratense</i>	<i>TrifPrat</i>				+			r	+	+		+																+	1			
<i>Trifolium rubens</i>	<i>TrifRube</i>		1	1		+	+	+		+			+	+	+		+						+	+								
<i>Veronica barrelieri</i>	<i>VeroBarr</i>								+							+							+									
<i>Vicia cracca</i>	<i>ViciCrac</i>								+				+																			

Relevés numbers in Table 1 correspond to the following relevés from the original table: No. 1–30: Kaligarč, 1997: Tab. 1, relevés No. 1, 2, 5, 7, 10, 12, 14, 16, 18, 19, 20, 21, 23, 24, 25, 27, 29, 30, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42 and 43.

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## DEVELOPMENT OF THE IDENTIFICATION KEY FOR REFERENCE FIG (*FICUS CARICA* L.) VARIETIES FROM SLOVENE ISTRIA

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### ABSTRACT

*In Slovenia, the common fig is an underutilized fruit species grown in family yards in association with olives and other Mediterranean plants. Due to the functional properties of fig syconias, the interest in fig cultivation is increasing. In order to provide certified plant material in the region of Slovene Istria, the determination of reference varieties is required on the basis of the molecular marker system. The identification key containing the minimum primer pairs for unambiguous discrimination of reference fig varieties was developed with the choice of the most informative microsatellite loci. All recommended fig varieties for Slovenia were genetically differentiated by only 3 loci: FCUP008-2, FCUP013-7, and FCUP044-6.*

**Key words:** *Ficus carica* L., reference variety, microsatellites, Slovene Istria

## SVILUPPO DELLA CHIAVE DI IDENTIFICAZIONE PER LE VARIETÀ REFERENZIALI DI FICO COMUNE (*FICUS CARICA* L.) DELL'ISTRIA SLOVENA

### SINTESI

*In Slovenia il fico comune è una specie fruttifera sottoutilizzata che cresce in orti di famiglia, in associazione con gli ulivi e altre piante mediterranee. Vista l'elevata concentrazione di vitamine, minerali e biofenoli nei frutti di fico, l'interesse per la coltivazione di tale pianta è crescente. Al fine di fornire materiale vegetale certificato nella regione dell'Istria slovena, la determinazione delle varietà referenziali è richiesta con l'ausilio di sistemi molecolari di marker. La chiave di identificazione permette una discriminazione non ambigua delle varietà referenziali di fico, ed è stata sviluppata con la scelta dei loci microsatellitari più informativi. Tutte le varietà di fico raccomandate per la Slovenia sono state geneticamente differenziate da soli 3 loci: FCUP008-2, FCUP013-7 e FCUP044-6.*

**Parole chiave:** *Ficus carica* L., varietà referenziali, microsatelliti, Istria slovena

## INTRODUCTION

The common fig (*Ficus carica* L.) is a typical fruit species of warm climate, widely spread in the Mediterranean Basin where its production is of great economic significance. In Slovenia, the cultivation of the common fig is limited to the coastal region of Slovene Istria, Goriška Brda and the Vipava Valley. Approximately 4,000 fig trees are planted on around 10 hectares of intensive orchards, and the production of fruits is estimated to total 50 tones per year (FAOSTAT, 2008). Yet large fig orchards are rare, and individual trees are frequently planted in family yards and gardens in association with olives and other Mediterranean plants. Fig fruits are prevalently sold fresh at local markets. The economic potential of fig cultivation has been poorly addressed, and the fig tree is considered an underutilized fruit species in the region. Over the last few years, increasing attention and promotion have been paid to underutilized species whose reemergence could lead to higher agricultural diversification, greater use of marginal lands and a more balanced diet (IPGRI, 2002).

The functional properties of fig fruits, such as richness of fibers, biophenols, vitamins and minerals, contribute to their great demand among the consumers and encourage the use of fig fruits in the food-processing industry. In order to encourage fig growing in Slovenia, the research on variety structure and an inventory of fig genetic resources are required. Morphological survey was initiated at the national level, and preliminary results showed a great diversity of fig varieties (Podgornik *et al.*, 2008). The efficiency of fig growing revitalization is greatly influenced by variety choice, since not all varieties are suitable for commercial use. The following fig varieties are recommended for the Slovene cultivation area: 'Bela petrovka' and 'Miljska figa' as major varieties, and 'Zuccherina', 'Zelenka', 'Flazana', 'Pinčica', 'Laščica', 'Sivka' as minor varieties (Godec *et al.*, 2007). Cuttings for fig tree propagation are usually collected from individual trees at different locations, therefore mother trees ought

to be determined and the identification system developed for propagation of certified plant material.

The objectives of the certification process are production and distribution of high quality plant material of superior varieties of established genetic identity and propagation of virus free plants. Even if morphological evaluation of varieties is traditional and well adopted, the use of phenotypic characteristics in the identification process is uncertain due to their high variation and dependence on environmental factors. Introduction of DNA molecular markers into the identification scheme of plant material has become a routine analytical approach, and among available marker systems, microsatellites or simple sequence repeats (SSR) are the most employed.

The recently developed microsatellites in the common fig (Khadari *et al.*, 2001; Giraldo *et al.*, 2005; Bandelj *et al.*, 2007) enabled introduction of these markers into the genotyping analysis of varieties. DNA fingerprinting of plants with microsatellites is reliable and fast once the most informative loci have been selected. Other advantages of the microsatellite marker system are the generation of non complex banding patterns, high polymorphism, and genotyping results comparable among different laboratories.

The aim of this work was to establish the identification key for recommended fig varieties in Slovenia by using microsatellite genotyped data. The identification key will serve for testing and confirmation of genetic identity of mother plants grown in nurseries for the purpose of propagation. The results of this work can serve as the basis for the establishment of the national fig collection and will be also used for fig genetic resources management in Slovenia.

## MATERIALS AND METHODS

## Plant material

The genotyping analysis included 8 recommended fig varieties for the Slovene cultivation area. From 1 to 4 fig

**Tab. 1: List of fig varieties included in the genotyping analysis, number of analysed samples and location of sampling.**

**Tab. 1: Seznam sort fig, vključenih v genotipizacijo, število analiziranih vzorcev in lokacija vzorčenja.**

Variety name	No. samples	Sampling location
'Bela petrovka'	4	Izola, Lucija, Glem, Dekani
'Miljska figa'	4	Seča, Osp, Glem, Dekani
'Zuccherina'	1	Šalara
'Zelenka'	1	Padna
'Pinčica'	1	Seča
'Laščica'	2	Padna, Nova vas
'Sivka'	1	Smokvica
'Flazana'	1	Goriška Brda
<b>Sum of samples</b>	<b>15</b>	

trees were sampled per variety on the basis of previous morphological description (Vrhovnik & Kodrič, 2004; Podgornik *et al.*, 2008). Fig leaves were collected from individual trees at different locations in Slovene Istria and Goriška Brda. The names of genotyped fig varieties and locations of sampled plant material are listed in Table 1.

### Fig DNA isolation and amplification of microsatellites

Fig DNA was extracted from leaves by modified CTAB method, and amplification of microsatellites with fluorescence-based detection was performed as previously reported by Bandelj *et al.* (2007). Four primer pairs for fig microsatellite loci (FCUP008-2, FCUP013-7, FCUP044-6, FCUP068-1) were used in the genotyping analysis. For fluorescent detection, short primers of the developed pair were elongated for the M13(-21) 18 bp sequence according to Schuelke (2000). Amplification reactions were carried out in a total volume of 10 µl, containing 20 ng of fig DNA, 1X supplied PCR buffer (Promega), 0.2 mM of each dNTP, (Roche), 0.25 unit of *Taq* DNA polymerase (Promega), 0.2 µM of each locus specific primer and 0.075 µM of M13(-21) primer labelled at the 5' end with Cy5 (MWG Biotech). Amplification was performed in a GeneAmp 9700 thermal cycler (Applied Biosystems), and the conditions of the two-step PCR amplification were as follows: 94 °C (5 min), then 5 cycles of 45 s at 94 °C, 30 s at the initial annealing temperature (57 °C for loci FCUP008-2 and FCUP013-7, 60 °C for loci FCUP044-6 and FCUP068-1), which was lowered by 1 °C in each cycle, and the extension at 72 °C for 1 min 30 s. The second step of amplification passed through 25 (FCUP008-2, FCUP013-7, FCUP044-6) or 28 (FCUP068-1) cycles with the same cycling conditions except for the constant annealing temperature of 52 °C (FCUP008-2, FCUP013-7) or 55 °C (FCUP044-6, FCUP068-1). The reactions ended by 8 min extension at 72 °C. The amplification products were separated on a 7.5% polyacrylamide denaturing gel, containing 7 M urea. Electrophoresis was performed on an automated ALFexpressII sequencer (Amersham Biosciences), and the length of alleles was determined with the aid of an external standard (50–500 bp, GE Healthcare) and internal standard using Allele Locator 1.03 software.

### RESULTS

In order to establish the database of reference fig varieties for Slovenia, genotyping with microsatellite mark-

ers was performed. The database of genotyped varieties was used as the basis for development of the identification key that includes the minimum primer pairs for distinguishing all varieties in case of propagation of certified plant material in nurseries. Development of the identification key enables fast and reliable determination of identity of mother plants, cuttings and young plants. For DNA genotyping analysis, 4 published microsatellite loci (FCUP008-2, FCUP013-7, FCUP044-6, FCUP068-1) were chosen (Bandelj *et al.*, 2007).

The microsatellite markers were successfully amplified in all 15 samples with 4 primer pairs used. PCR products were separated by polyacrylamide gel with high resolution using ALFexpressII sequencing instrument and detected automatically by fluorescence. Microsatellites lengths were determined automatically using a computer software package.

Altogether, 17 alleles were amplified at 4 loci in 8 fig varieties. 6 alleles were observed at locus FCUP008-2, 4 alleles were amplified at loci FCUP044-6 and FCUP068-1, and only 3 alleles were found at locus FCUP013-7. The varieties 'Bela petrovka', 'Miljska figa' and 'Laščica', represented by 4 and 2 samples respectively, showed identical DNA profiles, and no intra-variety polymorphism was found. The number of observed genotypes was 17, the highest number (5) was observed at locus FCUP008-2, while 4 different genotypes were found at other three loci. At 4 analyzed loci, 6 unique genotypes were observed. They were characteristic of the following varieties: 'Bela petrovka' [AE(160:180)], [NR(178:206)], 'Miljska figa' [BF(162,184)], 'Zuccherina' [PR(198:206)], 'Laščica' [DF(178:184)], and 'Flazana' [HH(208:208)] (Tab. 2).

Variety specific or unique alleles were amplified in two varieties, 'Bela petrovka' (allele A: 160 bp at locus FCUP008-2) and 'Zuccherina' (allele P: 198 bp at locus FCUP068-1).

The allelic polymorphism allowed the discrimination of all analyzed varieties. The presence of individual alleles generated by 4 primer pairs in 8 fig varieties is shown in Table 2. A minimum number of 3 microsatellite markers were chosen for rapid varietal identification of recommended fig varieties. Specific allele profiles at locus FCUP008-2 were first assigned to three varieties: 'Bela petrovka', 'Miljska figa' and 'Laščica', the next three varieties, 'Sivka', 'Flazana' and 'Zuccherina', were differentiated by FCUP013-7, and the remaining two varieties, 'Zelenka' and 'Pinčica', were additionally genotyped by FCUP044-6. The identification key for the 8 fig varieties is presented in Table 2.

**Tab. 2: Identification of 8 recommended fig varieties in Slovenia by microsatellite markers. The presence of alleles in specific genotype/variety is marked by symbol +. All varieties were differentiated by 3 microsatellite loci: FCUP008-2, FCUP013-7, FCUP044-6, which form the molecular identification key for fig varieties.**

**Tab. 2: Identifikacija 8 priporočenih sort fig v Sloveniji z mikrosatelitskimi markerji. Zastopanost alelov v specifičnem genotipu/sorti je označena s simbolom +. Vse sorte ločimo s tremi mikrosatelitskimi lokusi: FCUP008-2, FCUP013-7, FCUP044-6, ki oblikujejo molekularni identifikacijski ključ za sorte fig.**

Locus	Allele designation and allele in bp		'Bela petrovka'	'Miljska figa'	'Zuccherina'	'Zelenka'	'Pinčica'	'Laščica'	'Sivka'	'Flazana'
FCUP 008-2	A	160	+							
	B	162		+	+	+	+			
	C	166							+	+
	D	178						+	+	+
	E	180	+		+	+	+			
	F	184		+				+		
FCUP 013-7	G	196				+	+	+	+	
	H	208						+	+	++
	I	212	++	++	++	+	+			
FCUP 044-6	J	208					+	+	+	+
	K	210							+	+
	L	217		++		++				
	M	219	++		++		+	+		
FCUP 068-1	N	178	+						+	+
	O	196		++		++	++	++	+	+
	P	198			+					
	R	206	+		+					
Observed genotypes			AE, II, MM, NR	BF, II, LL, OO	BE, II, MM, PR	BE, GI, LL, OO	BE, GI, JM, OO	DF, GH, JM, OO	CD, GH, JK, NO	CD, HH, JK, NO

## DISCUSSION AND CONCLUSIONS

In the Mediterranean Basin, the cultivated fig is a widely spread fruit species with a large number of local varieties whose identity has been poorly studied. The identification of reference varieties is important especially to nurseries where certified plant material is propagated. The accurate identification system of varieties is also important to growers when they plan to establish new permanent orchards as the quality of the crop is greatly influenced by variety choice. A reliable identification technique of plant material is also of significant importance for the establishment of germplasm collection and prevention of plant mislabelling during plantation. Long juvenile stage of vegetatively propagated fig trees and strongly expressed heterophylly prevent the determination of young trees' identity on the basis of morphological characteristics. The development of DNA markers has brought about new approaches in varietal analysis. DNA based identification procedures enable plant identification by generating genotype specific DNA banding profiles. Among available DNA markers, randomly amplified polymorphic DNA (RAPD)

has been the most employed in *Ficus carica* L. germplasm characterization (De Masi *et al.*, 2005; Salhi-Hannachi *et al.*, 2005; Sadler & Ateyyeh, 2006), and the recent development of fig microsatellites enabled the inclusion of these markers into diversity studies of common figs (Khadari *et al.*, 2001; Giraldo *et al.*, 2005; Bandelj *et al.*, 2007).

In order to establish an accurate identification system for recommended fig varieties in Slovenia, DNA profiling of 15 fig samples belonging to 8 reference varieties was performed. Among the 3 published sets of fig microsatellite markers, we chose 4 FCUP primer pairs on the basis of their good polymorphic characteristics exhibited in our previous diversity study of cultivated figs (Bandelj *et al.*, 2007). The highest number of amplified markers was displayed by loci FCUP008-2, FCUP044-6, FCUP068-1 and FCUP70-2, with the criteria for marker choice also being high polymorphic information content value and low probability of identity. The choice of the most appropriate markers is an important step in the identification procedure of varieties as it affects the rapidity and extent of molecular analysis and, consequently, the cost of the procedure.

The selected microsatellite loci allowed the discrimination of all 8 fig varieties. The average number of amplified alleles per locus was 4.25, thus being identical to the number reported by Khadari *et al.* (2001) in their diversity study of 14 fig varieties. Using LMFC set of microsatellites, Giraldo *et al.* (2005) detected a lower number of alleles per locus (3) in their genotyping analysis of 15 fig varieties. The number of detected alleles is probably dependent on characteristics of loci and genetic background affecting similarity of varieties included into the analysis. In our study, high genetic similarity was observed between the varieties 'Flazana' and 'Sivka', as well as between 'Zelenka' and 'Pinčica'. Differences in allelic profiles of the above-mentioned varieties were found only at loci FCUP013-7 and FCUP044-6 respectively, while identical alleles were observed at other three loci.

The comparison of allelic profiles of samples within the varieties 'Bela petrovka', 'Miljska figa' and 'Laščica' revealed no differences, which testified to the homogeneity of the analyzed varieties and the absence of different clones. In other studies, genetically heterogeneous varieties were identified by means of inter simple sequence repeat, microsatellites (Khadari *et al.*, 2005), RAPDs and AFLPs (Cabrita *et al.*, 2001).

Discrimination of varieties by DNA markers is usually performed in the following three ways: by using unique markers, unique genotypes, and the combination of DNA profiles in different DNA regions. Our study revealed only 2 unique alleles characteristic of the varieties 'Bela petrovka' and 'Zuccherina'. These two varieties could be immediately identified by genotyping loci FCUP008-2 and FCUP068-1. Unique genotypes were found at all loci except at locus FCUP044-6 where two

by two varieties shared the same allelic profile. By applying the combination of 3 loci (FCUP008-2, FCUP013-7 and FCUP044-6), we were able to distinguish all 8 reference fig varieties. Giraldo *et al.* (2005) distinguished 9 fig varieties by only 2 microsatellite loci. The number of primer pairs required for the discrimination analysis depends on genetic background and relatedness of analyzed varieties. The choice of locus is also an important factor in the discriminating process. In our previous study, locus FCUP068-1 generated high variability information (Bandelj *et al.*, 2007), while in the present analysis it revealed identical genotypes for the varieties 'Miljska figa', 'Zelenka', 'Pinčica' and 'Laščica'. On the basis of this result, it can be concluded that locus FCUP068-1 is not suitable for variety identification of Slovene reference figs.

The present study provides the first identification key that includes the minimum number of primer pairs required for unambiguous discrimination of recommended fig varieties in Slovenia. Microsatellites proved to be a valuable molecular tool for varietal identification of figs, and the results of this work could be successfully implemented for confirmation of varietal identity of propagated fig plants in nurseries, for identification of fig trees of unknown identity in Slovene cultivation area, as well as for screening and managing of fig genetic resources in collections.

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## RAZVOJ IDENTIFIKACIJSKEGA KLJUČA ZA REFERENČNE SORTE FIG (*FICUS CARICA* L.) SLOVENSKE ISTRE

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#### POVZETEK

Figa je v Sloveniji poznana kot manj razširjena in uporabljena sadna vrsta. V Slovenski Istri in toplejših predelih Vipavske doline in Goriških Brd uspeva na družinskih vrtovih v asociaciji s tipičnimi sredozemskimi kulturami. Visoka vsebnost vlaknin, biofenolov, vitaminov in mineralov v plodovih fige prispeva k njenemu večjemu povpraševanju na trgu ter k vključevanju sadežev v živilsko-predelovalno industrijo. Za pospeševanje gojenja fig v Primorju je pomembno zagotoviti sadilni material referenčnih in priporočenih sort. Z razvojem molekulskih markerjev identiteto sort v sadjarstvu ugotavljamo na nivoju genoma. Namen razvoja molekulskih identifikacijskih ključev v

sadjarstvu je določiti visoko informacijske molekulske markerje za posamezno sadno vrsto, s katerimi po opravljeni genotipizaciji sorte genetsko hitro ločimo, zmanjša pa se tudi obseg laboratorijskih analiz. Referenčne sorte fig, ki so predstavljene v sadnem izboru za Slovenijo, smo ločili s tremi visoko informativnimi lokusi mikrosatelitov (FCUP008-2, FCUP013-7 in FCUP044-6), ki ponazarjajo identifikacijski ključ oziroma najmanjše možno število lokusov, na osnovi katerih lahko priporočene sorte fig genetsko ločimo.

**Ključne besede:** *Ficus carica* L., referenčna sorta, mikrosateliti, Slovenska Istra

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## PERIPHYTON BIOINDICATORS IN THE SITNICA RIVER (KOSOVO)

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### ABSTRACT

*Periphyton consists of benthic algae that grow attached to surfaces of rocks or larger plants. The aim of the study was to investigate the periphyton of the Sitnica River between Kuzmin and Lumëmadhë (Kosovo). Altogether, 37 algal taxa were determined. The dominating group consists of diatoms (Bacillariophyta). According to bioindicators in Sitnica water, the examined parts could be classified into quality categories III – II and III; they belong to the alpha – beta mesosaprobic and alpha-mesosaprobic levels.*

**Key words:** Sitnica River, periphyton, algae, Bacillariophyta, water saprobicity

## PERIFITON QUALE BIOINDICATORE NEL FIUME SITNICA (KOSOVO)

### SINTESI

*Il perifiton è composto da alghe bentoniche che crescono attaccate a substrati rocciosi o piante più grandi. Scopo dello studio era quello di studiare il perifiton del fiume Sitnica fra le località di Kuzmin e Lumëmadhë (Kosovo). Sono stati determinati 37 taxa, fra i quali dominavano le diatomee (Bacillariophyta). In base ai bioindicatori nel fiume Sitnica, i siti campionati possono venir classificati come categorie di qualità III – II e III, il che significa che appartengono ai livelli alfa-beta-mesosaprobici ed alfa-mesosaprobici.*

**Parole chiave:** fiume Sitnica, perifiton, alghe, Bacillariophyta, saprobietà dell'acqua

## INTRODUCTION

Periphyton is the mixture of algae, bacteria and fungi that grows on rocks, snags, macrophytes and man-made structures in streams (Rutherford & Cuddy, 2005). It is an important food source at the base of the food web and makes a positive contribution to the ecosystem health. However, it can adversely affect aesthetics and ecosystem health if its biomass is excessive and/or its metabolism causes very large diurnal fluctuations of pH and dissolved oxygen concentration (Rutherford & Cuddy, 2005).

Aquatic organisms can serve as indicators of the properties of the surrounding environment. They are applied mainly in the field of water quality and in its central part saprobity (Sladeckova & Sladecek, 1993). Saprobity describes the effects of the content of putrescible organic matter undergoing microbial decomposition. The common processes of eutrophication, pollution, degradation and selfpurification can be damaged or destroyed by toxic, radiochemical and some physical factors interfering with saprobity. Biological indicators enable us to distinguish individual saprobic levels by microscopical analysis (Sladeckova & Sladecek, 1993).

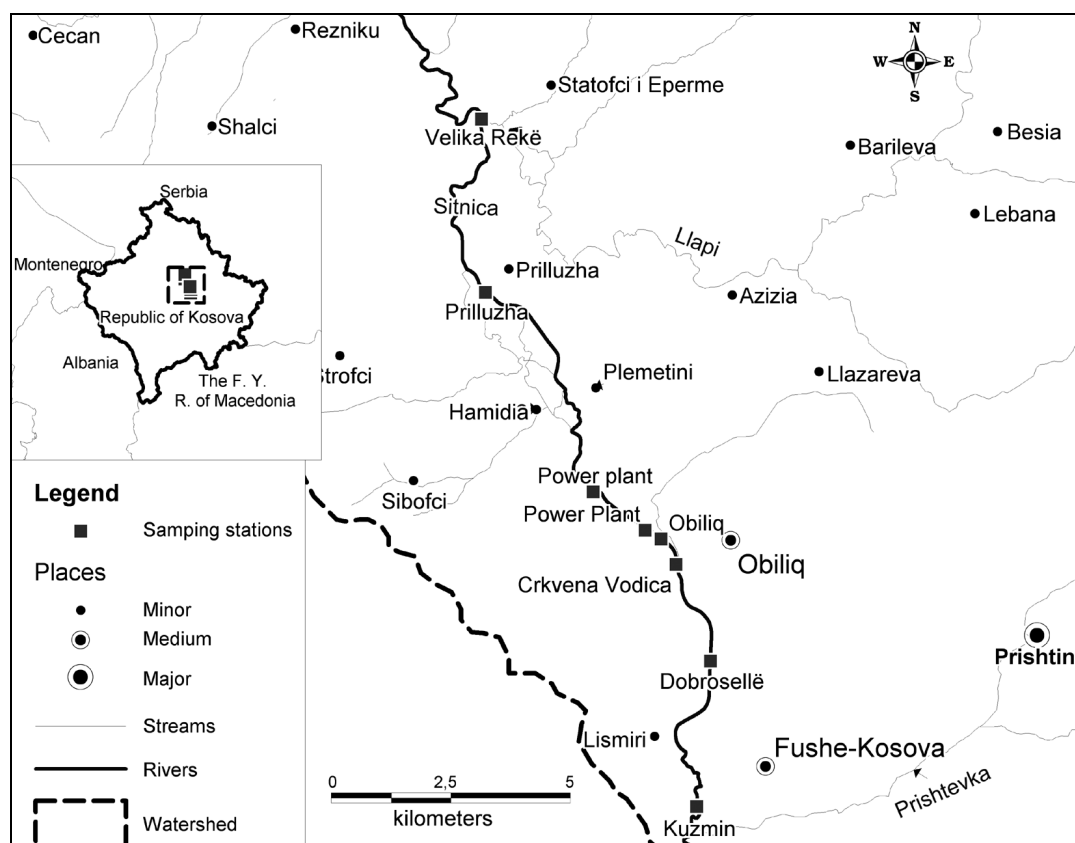
The Sitnica River flows through Kastriot (Obilic), where thermo power plants are located. Sitnica is the main collector for pollution, derived from the "Kosova" power plant, the town of Kastriot, and from some villages situated along or near the Sitnica River. Suspended crumbs like carbonic dust, which is released from "Kosova" power plant, silt up the bottom of the river and causes transformation not only in the structure of substrate but also in the biocenosis.

The aim of this investigation was to analyze the periphyton (phytobenthos) in the Sitnica River in order to determine the level of organic pollution in it, which develops under the influence of specific environmental factors.

## MATERIAL AND METHODS

## Study site

The Sitnica River is 154 km long, with its source located near the village of Vragolia (Fig. 1). The Sitnica joins the Ibri River in the northern part of Kosovo. Summers are warm and dry, while winters are cold and rainy. Annual average air temperature reaches 16.7 °C.



**Fig. 1: The map of the Sitnica River with sampling stations.**

**Sl. 1: Zemljevid reke Sitnice z vzorčišči.**

Average temperature during the coldest month (January) is 2.3 °C and 23.2 °C during the warmest month (July). The highest rainfall is recorded between October and March (data from the Meteorological and Hydrological Service of the Republic of Kosovo), the lowest between June and August. The water level of the Sitnica River varies from 45–90 cm.

Sampling localities:

1 - Right bank of the Sitnica River after the village of Kuzmin, ahead of the flow of effluents from the "Kosova" power plant;

2 - Right bank of the Sitnica River, downstream of the inflow of the effluents from the "Kosova" power plant at Kastrioti;

3 - Right bank of the Sitnica River under the bridge leading to Dobro Selo;

4 - Right bank of the Sitnica River downstream of the inflow of fenolic waste waters from the "Kosova" power plant;

5 - Opposite to locality 4;

6 - Right bank of the Sitnica River at the bridge leading to Crkvene Vodice;

7 - Right bank of the Sitnica River near the village of Prilluzh;

8 - Right bank of the Sitnica River downstream of the village of Lumëmadhë, 600 m after joining the Llap River.

### Experimental procedure

Material was obtained by collecting sediments and stones taken from the river bottom (10–30 cm deepness) at five stations between Kuzmin and Lumëmadhë. The collected material was fixed with 4% formaldehyde. Phytomicrobenthos was examined with Leica microscope. Determination of algae protocol followed Geitler (1932), Gollerbah et al. (1935), Zabelina et al. (1951) and Lazar (1960). The saprobity levels were determined by Sladeczek (1973). Evaluation of saprobity was carried out on the basis of indicator species and standard procedure of Pantle & Buck (1955), Knopp (1954–1955) and Krammer & Lange Bertalot (1986–1991).

The relative abundance of phytoplankton was determined according to the modified sixth degree scale (Kawecka, 1980).

### Cleaning of diatoms

Cleaning of diatom frustules, preparation of permanent slides and determinations follow Krammer & Lange Bertalot (1986–1991). The analyzed water was put into a 600 ml glass beaker with 20 ml of concentrated HNO<sub>3</sub>. The beaker was placed on a hotplate and heated until the volume of liquid was reduced to about 20 ml. From time to time, the particles of K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> were added. The

samples were finally rinsed with tap water, until reaching 7 pH.

## RESULTS AND DISCUSSION

The greatest numbers of algal taxa were registered at localities 6 and 8 (20 species each) (Tab. 1). Locality 6, which was 1 km downstream of locality 4, was relatively unpolluted, especially in comparison with locality 4 (where toxic phenol waters are released), perhaps as the result of high level of water that diluted the waters with high concentration of phenol from the "Kosova" power plant. When the water level was low, the influence of sewage with phenol on development of phytomicrobenthos was noticed much further down the stream, as shown by some earlier investigation (Maloseja & Gecaj, 1984). 600 m above locality 8, the Llap River joins the Sitnica, which helps in the regeneration of ecological condition in the Sitnica River and improves its algal richness.

At localities 2 and 4, low numbers of algae and their dead cells were perceived (Tab. 1), since the "Kosova" power plant spills the effluent waters into the Sitnica River (Mackenthun, 1970; Burrous, 1971; Ivora et al., 2000; Megharaj et al., 2004).

The phytomicrobenthos conditions at locality 3 indicate weakness of toxic effect of high alkaline water (pH 12), lodges of ash and furnace after 600 m. We found some diatoms that dominate nearly in the entire part, particularly the following species: *Navicula viridula*, *Navicula exigua*, *Nitzschia aciculari*, *Nitzschia palea*, *Nitzschia paleacea*, *Nitzschia terminalis* var. *minor* and *Suirella ovata*. Their occurrence could be explained by optimal light rate and temperatures of Sitnica water in the summer, which are between 18–25 °C and represent an optimum for the growing and development of this group of algae (Whitford, 1968; Habdija, 1970; Grin, 1971; Oksijuk, 1973; Maloseja, 1979). At nearly all localities, the presence of green flagellate alga of the genus *Euglena* was noticed, which is a sign of high organic load through the entire research area.

The use of bioindicator species is considered to have advantages over intermittent chemical analyses, as organisms integrate environmental influences. The qualitative and quantitative composition of biocenosis varied depending on organic pollution, toxic substance and locations.

The fact is that in the examined part of Sitnica, life is developing under the influence of specific ecological factors. According to the saprobiological analysis on the basis of indicator species of "lower plants" in phytobenthos, the researched part of the Sitnica river belongs to quality classes III – II, respectively alphamesosaprob and alpha – beta – mesosaprob (Tab. 2).

Tab. 1: List of the algal taxa determined in the Sitnica River during the summer of 2005. 37 species of cyanobacteria and algae from divisions Cyanophyta (1), Euglenophyta (2) and Bacillariophyta (34) are listed.

Legend: o – oligosaprobic level;  $\beta$  – beta-mesosaprobic level;  $\alpha$  – alpha-mesosaprobic level; p – polisaprobic level; 1, 2, 3, 4, 5, 7 – relative abundance. The relative abundance of the phytoplankton has been determined according to modified sixth degree scale by Kawecka (1980).

Tab. 1: Seznam taksonov alg, ugotovljenih poleti 2005 v reki Sitnici (Kosovo): 37 vrst cianobakterij in alg iz odredkov cianofitov (1), evglenofitov (2) in bacilariofitov (34).

Legenda: o – oligosaprobna stopnja;  $\beta$  – beta-mezosaprobna stopnja;  $\alpha$  – alpha-mezosaprobna stopnja; p – polisaprobna stopnja; 1, 2, 3, 4, 5, 7 – relativna abundanca. Relativna gostota fitoplanktona je bila ugotovljena v skladu z modificirano lestvico šeste stopnje (Kawecka, 1980).

Taxa	Saprobic level	Locality							
		1	2	3	4	5	6	7	8
No. species per locality		29	10	11	5	20	24	24	33
Cyanophyta									
<i>Oscillatoria putrida</i> (Smith)	p	-	-	-	-	1	-	-	-
Bacillariophyta									
<i>Caloneis amphisbaena</i> (Cl.)	$\beta$ - $\alpha$	1	-	-	-	-	2	2	3
<i>Cymbela austriaca</i> (Grun)	-	2	-	-	-	-	1	1	3
<i>C. ventricosa</i> (Kütz)	$\beta$	1	1	-	-	-	1	-	2
<i>C. affinis</i> (Kütz)	o- $\beta$	1	-	-	-	-	-	-	1
<i>Cymatoplura solea</i> (W. Smith)	$\beta$ - $\alpha$	1	-	-	-	-	-	-	1
<i>Diatoma vulgare</i> (Bory)	$\beta$	-	-	-	-	1	1	1	1
<i>D. elongatum</i> var. <i>tenuis</i>	-	1	-	1	-	-	1	-	1
<i>Gyrosigma acuminatum</i> (Raben.)	$\beta$	1	-	-	-	1	1	1	1
<i>G. scalpoides</i> (Cleve)	-	-	-	-	-	-	-	1	1
<i>Gomphonema olivaceum</i> (Kütz)	$\beta$	1	-	-	1	1	-	-	1
<i>Hantzschia amphioxys</i> (Grun)	$\alpha$	-	-	1	-	-	1	1	-
<i>Navicula gracilis</i> (Ehr)	$\beta$ -o	-	-	-	1	-	1	2	3
<i>N. cryptocephala</i> (Kütz)	$\alpha$	2	-	1	-	1	-	1	3
<i>N. exigua</i> (Muller)	$\beta$	5	-	4	-	5	3	3	2
<i>N. radiosa</i> (Kütz)	o- $\beta$	1	-	-	-	-	-	-	1
<i>N. rhynchocephala</i> (Kütz)	$\alpha$	-	1	-	-	-	-	-	1
<i>N. viridula</i> (Kütz)	$\alpha$	3	1	5	1	2	2	5	7
<i>Nitzschia acicularis</i> (W. Smith)	$\alpha$	3		5	-	2	2	-	3
<i>N. gracilis</i> (Hatzsch)	-	2	-	-	-	-	-	-	1
<i>N. hungarica</i> (Grun)	$\alpha$	1	1	-	-	-	-	1	2
<i>N. palea</i> (W. Smith)	$\alpha$	7	-	7	1	3	3	5	5
<i>N. paleacea</i> (Grun)	-	3	-	-	-	2	3	3	-
<i>N. recta</i> (Hatzsch)	$\beta$ - $\alpha$	-	-	-	-	1	-	-	1
<i>N. stagnorum</i> (Raben)	$\beta$	5	-	-	-	5	5	5	7
<i>N. sigmoidea</i> (W. Smith)	$\beta$	1	-	-	-	1	-	1	1
<i>N. termalis</i> var. <i>minor</i> (Hasle)	-	3	1	3	-	3	3	3	5
<i>N. vermicularis</i> (Grun)	$\beta$	2	1	-	-	3	3	3	1
<i>Pinnularia microstauron</i> var. <i>brebissoni</i> (Kütz)	-	3	3	-	-	1	3	3	5
<i>Roichosphaenia curvata</i> (Gr)	$\beta$	-	-	-	1	-	-	-	1
<i>Stauroneis anceps</i> (Ehr.)	-	1	-	-	-	-	1	1	1
<i>S. smithi</i> (Grunow)		1	-	-	-	-	1	1	3
<i>Synedra ulna</i> (Her)	-	1	-	1	-	-	1	1	2
<i>Surirella ovata</i> (Kütz)	-	2	1	1	-	1	2	1	1
<i>S. linearis</i> (W. Smith)		1	3	-	-	1	1	1	2
Euglenophyta									
<i>Euglena viridis</i> (Ehr)	$\beta$ -p	5	1	5	-	5	5		5
<i>E. sanguinea</i> (Ehr)	$\beta$	5	-	-	-	5	3	1	-

**Tab. 2: The evaluation of the saprobity of Sitnica waters. The saprobic index and saprobic level are according to the Pantle-Buck criteria (Pantle & Buck, 1955).**

**Tab. 2: Ocenjevanje saprobnosti vode v reki Sitnici. Saprobní indeks in saprobna stopnja sta v skladu s kriteriji Pantle-Buck (Pantle & Buck, 1955).**

Parameter	Locality							
	1	2	3	4	5	6	7	8
<b>Saprobic index</b>	2.73		2.83		2.83	2.61	2.80	2.65
<b>Saprobic level</b>	a-b		a		a	a-b	a	a-b
<b>Quality class</b>	III-II		III		III	III-II	III	III-II

The low quality classes stated on the basis of algalogical analyses correspond to class IV class determined by bacteriological and hydrochemical parameters for the same part of Sitnica (Plakolli et al., 1988).

Aquatic communities, both plant and animal, integrate and reflect the effects of chemical and physical disturbances occurring over extended periods of time.

## CONCLUSION

In the researched part of the Sitnica River, 37 algal taxa were determined. The highest number of taxa was found at localities 6 and 8 (20 species each). The dominating group consists of diatoms (Bacillariophyta). Based on the presence of species as saprobiologic bioindicator and saprobic level, the researched part of the Sitnica River could be classified into quality categories III – II and III.

## PERIFITONSKI BIOINDIKATORJI V REKI SITNICI (KOSOVO)

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## POVZETEK

Perifiton sestoji iz bentoških alg, ki poraščajo kamne, skale in večje rastline. Glavni namen pričujoče študije je bil preučiti perifiton reke Sitnice med krajema Kuzmin in Lumëmadhë (Kosovo). Skupaj je bilo ugotovljenih 37 taksonov. Prevladujočo skupino so oblikovale kremenaste alge (Bacillariophyta). Glede na bioindikatorje v reki Sitnici lahko njene pregledane dele vključimo v kakovostne razrede III – II in III, kar pomeni, da pripadajo alfa-beta-mezosaprobnim in alfa-mezosaprobnim stopnjam.

**Ključne besede:** reka Sitnica, perifiton, alge, Bacillariophyta, saprobnost vode

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## WORMLION *VERMILEO VERMILEO* (L.) (DIPTERA: VERMILEONIDAE) IN SLOVENIA AND CROATIA

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### ABSTRACT

*The occurrence of the wormlion species Vermileo vermileo (Linnaeus, 1758) is confirmed for the Mediterranean region of Slovenia and Croatia. Its predatory larvae were found in substrates consisting of fine sand or powder, where they construct pitfall traps similar to those of antlions. Wormlion larvae were reared in laboratory to study their biology.*

**Key words:** wormlion, *Vermileo*, Diptera, Slovenia, Croatia

## VERMILEONE, *VERMILEO VERMILEO* (L.) (DIPTERA: VERMILEONIDAE), IN SLOVENIA E CROAZIA

### SINTESI

*La presenza della specie chiamata vermileone, Vermileo vermileo (Linnaeus, 1758), è confermata per la regione mediterranea di Slovenia e Croazia. Le larve predatrici di tale specie sono state trovate in substrati composti da sabbia fine o polvere, dove esse costruiscono trappole a imbuto, simili a quelle dei formicaleone. Le larve di vermileone sono state allevate in laboratorio al fine di studiarne la biologia.*

**Parole chiave:** vermileone, *Vermileo*, Diptera, Slovenia, Croazia

## INTRODUCTION

Wormlions (Vermileonidae) constitute the only known dipteran family, whose larvae capture prey by constructing pitfall traps in loose soil (Fig. 1), similar to those in antlions (Neuroptera: Myrmeleontidae) (Wheeler, 1930; Ludwig *et al.*, 1996, 2001). This feature is unique among Diptera.

Adults ("wormflies") are small (about 5 mm long), slender, nearly bare flies with stylate antennae, slender legs, and long, slender abdomen. The wings are narrowed at the base, without an alula or a developed anal angle (Triplehorn & Johnson, 2005). Adults have a life span of only two to five days, and during this period they feed on nectar and mate. Eggs are laid under the surface of fine-grained sand or powder in which the larvae live.

Wormlion larvae have been described by Wheeler (1930) and Ludwig *et al.* (1996, 2001). They have a small, almost completely retracted head capsule. The wormlion larvae are specialized for prey capture, especially their mouthparts and pseudopodium (Ludwig *et al.*, 1996, 2001). The pseudopodium is probably involved in the mechanical detection and seizure of the prey. An abdominal comb composed of a transverse row of long spines is used to anchor the posterior part of the larval body in the sand (Ludwig *et al.*, 2001).

Larvae construct conically shaped pitfall traps in fine dry sands and powders at the bases of cliffs and other locations, protected against rainfall. When different substrates are available, the larvae prefer finest sands with the smallest sand particle size and avoid coarser sand (Devetak, 2008). The larvae build pits by throwing sand with their heads. Once the pit is complete, the larva positions its body across the bottom of the pit, ventral surface up, at or just below the surface where it waits for prey (Petersen & Baker, 2006). When an ant or another small arthropod falls into the trap, it is grasped by the larva, which consumes the prey's softer parts and then throws the carcass out of the pitfall trap. The larvae pupate and about a month later the adults emerge (Teskey, 1981; Petersen & Baker, 2006).

The vermilionids were once placed in the family Rhagionidae, but Nagatomi (1977) gave them family rank. Vermileonidae differ from the rhagionids in having the wings more narrowed at the base and in having apical spurs on the front tibiae (Triplehorn & Johnson, 2005). Griffiths (1994) established a new infraorder, Vermileonomorpha, for vermilionids as no certain relationships with other brachyceran families had been proved (see also Stuckenberg, 2004). Vermileonidae are distributed in the Mediterranean part of Europe, the Middle East, Asia, North and South Africa, and North and Central America (Ludwig *et al.*, 2001). About 55 species in 7 genera are known (Nagatomi *et al.*, 1999).



**Fig. 1: Pitfall traps of larvae of the wormlion *Vermileo vermileo* in loose soil in Baška Voda (Dalmatia, Croatia).**

**Sl. 1: Lijakaste pasti ličink črvastega volkca *Vermileo vermileo* v rahli prsti v Baški Vodi (Dalmacija, Hrvatska).**

In the monograph "Živalstvo Slovenije" (Fauna of Slovenia), Sivec *et al.* (2003) mention occurrence of a wormlion *Vermileo vermileo* in Slovenia without citing detailed location. The aim of the study is to provide detailed information on the species in the country and in the neighbouring regions of Croatia.

## MATERIAL AND METHODS

Low abundance of adults makes them difficult to collect, so I was focused on the wormlion larvae, which are easily collected by looking for the pits in loose sands and powders. Larvae were taken from their pits by excavating the sand containing the pits with a spoon, and then by sieving the substrate.

**Substrate particle size analysis.** Mechanical analysis of particle size distribution of substrates from natural habitats was conducted using standard sieves (for details of the method see Devetak, 2008).

**Rearing.** The wormlion larvae were kept in the laboratory, at room temperature, in plastic containers (60 x 45 x 10 cm) filled with sand from natural habitats. Substrate particle size was  $\leq 0.23$  mm. Workers of the ant species *Lasius emarginatus* (Olivier) were used as food source for the wormlions. Feeding took place every day and one ant was delivered to each pit.

## RESULTS

***Vermileo vermileo* (Linnaeus, 1758)**Material examined

Slovenia:

Osp: 11. VI. 2005, 3 larvae

Piran: 15. VII. 2005, 1 larva; VI. 2006, 3 larvae

Fiesa: 22. V. 2008, 7 larvae

Croatia:

Rovinj: Aquarium, 9. VI. 2005, 4 larvae; 3. VI. 2008  
7 larvae

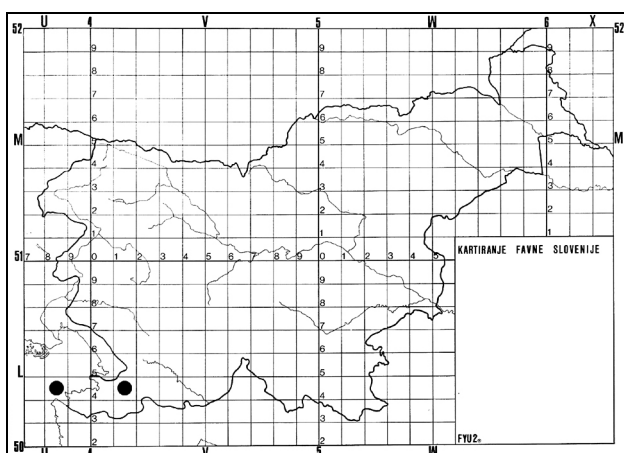
Premantura: 20. VII. 1984, 1 larva

Lošinj: Sv. Jakov, VI. 2000, 2 larvae

Podaca: VII. 2007, 3 larvae

Baška Voda: VIII. 2006 10 larvae; 2 adults emerged  
in the laboratory on 10. VI. 2007

All individuals were collected by the author.



**Fig. 2: Distribution of the wormlion *V. vermileo* in Slovenia.**

**Sl. 2: Razširjenost črvastega volkca *V. vermileo* v Sloveniji.**

The distribution of wormlions in Slovenia is shown in figure 2. The larvae built their pits in sands or powders in crevices of stone walls (Piran, Osp; Fig. 3), in fine substrate at the bases of cliffs (Baška Voda, Podaca, Sv. Jakov on the island of Lošinj) or in places under tree crowns protected against rainfall (Fiesa, Rovinj). In Osp, sieving analysis revealed the following particle size composition of the substrate (expressed in weight %): 14.5% represent fraction with particle size  $\leq 0.06$  mm; 17% fraction 0.06–0.11 mm; 11.5% fraction 0.11–0.23 mm; 36% fraction 0.23–0.54 mm; the rest fractions 0.54–2.2 mm (see also Devetak, 2008).

## DISCUSSION

Occurrence of wormlions in Slovenia has been confirmed for the Sub-Mediterranean region. Larvae were often found in substrates in crevices of stone walls, rarely in places under tree crowns. In natural habitats, the wormlion larvae construct their pits in finer substrates than antlion *Euroleon nostras* larvae.

Devetak (2008) tested substrate particle size-preference of *Vermileo vermileo* larvae. The pit-building decision of the larvae of the wormlions was observed in four substrates consisting of different sand fractions (particle sizes:  $\leq 0.23$  mm; 0.23–0.54 mm; 0.54–1 mm; 1–1.54 mm). Wormlions preferred the finest sand fraction ( $\leq 0.23$  mm) and avoided two coarser fractions (0.54–1 mm; 1–1.54 mm) (Devetak, 2008).

In Piran, Rovinj and Baška Voda, co-existence of wormlions and antlions *Euroleon nostras* was noted (Devetak, 2008). In experiments when two species were placed in the same container with two different substrates, interspecific predation did not occur, but cannibalism in antlions did. In natural habitats, cannibalism in antlions is often recorded (Devetak, 2000) and other insects also prey on antlions. While wormlion larvae readily build pits in the finest sands, antlion larvae prefer coarser sands. Wormlion preference for the finest sands and powders, and antlion preference for sands of medium particle size was confirmed by field observations (Devetak, 2008). In the field it seems that antlions avoid areas inhabited by wormlions, and vice versa. Wormlions and antlions may simply prefer different substrates independent of the presence of heterospecifics.



**Fig. 3: Habitat of wormlions in crevices of stone walls in the village of Osp (Slovenia).**

**Sl. 3: Habitat črvastega volkca v špranjah kamnitega zidu v Ospu (Slovenija).**

## ACKNOWLEDGEMENTS

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## ČRVASTI VOLKEC *VERMILEO VERMILEO* (L.) (DIPTERA: VERMILEONIDAE) V SLOVENIJI IN NA HRVAŠKEM

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## POVZETEK

Potrjeno je pojavljanje črvastega volkca *Vermileo vermileo* (Linnaeus, 1758) v mediteranskem območju Slovenije in Hrvaške. Predatorske ličinke živijo v substratih iz drobnega peska ali praška, v katerih gradijo lijakaste pasti, podobno kot volkci. Avtor je gojil ličinke črvastega volkca, da bi spremljal njihovo biologijo.

**Ključne besede:** črvasti volkec, *Vermileo*, dvokrilci, Slovenija, Hrvaška

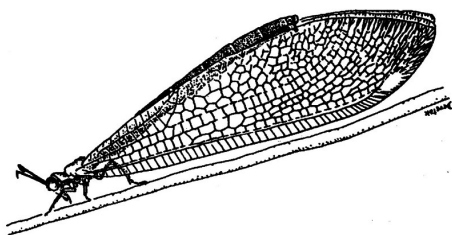
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**DELO NAŠIH ZAVODOV IN DRUŠTEV  
ATTIVITÀ DEI NOSTRI ISTITUTI E DELLE  
NOSTRE SOCIETÀ  
ACTIVITIES BY OUR INSTITUTIONS  
AND ASSOCIATIONS**

10<sup>TH</sup> INTERNATIONAL SYMPOSIUM ON  
NEUROPTEROLOGY, PIRAN, 22-25 JUNE 2008

X International Symposium on Neuropterology



22-25 June 2008, Piran, Slovenia

From June 22 to 25, 2008, the 10<sup>th</sup> International Symposium on Neuropterology was held in Piran. The Symposium was organized by Department of Biology of the Faculty of Natural Sciences and Mathematics, Maribor University, together with Marine Biology Station in Piran and under the patronage of the Slovenian Museum of Natural History and Slovenian Entomological Society "Štefan Michieli". More information is provided on the URL address [www.sympneuro.net](http://www.sympneuro.net). The meeting was held in a relaxed and friendly atmosphere in the northernmost part of the Adriatic Sea, in Barbara Fiesa Hotel, Piran.

The organizers brought together neuropterists from

around the world who are interested in any aspect of the neuropterid insects study (Neuropterida: Megaloptera, Raphidioptera, Neuroptera). The Symposium hosted 55 participants from twenty countries worldwide (Europe, Africa, Asia, North and South Americas). The official language of the Symposium was English.

Thirty-seven oral presentations were included in the following sessions: Digital initiatives in the Neuropterida; Taxonomy and systematics; Biology and ecology; History of Neuropterology; Biology of Chrysopidae; Cell biology; Biology of the antlion larvae; Phylogeny and systematics; Biogeography. In two poster sessions, 22 contributions were presented from various areas of Neuropterology. Participants presented the recent findings of their research, including new methods in morphological studies, exploration of previously almost unknown geographic areas, analysis of molecular biology data, and study of learning processes. The accompanying 41-page Abstract Book comprises scientific program, abstracts of oral and poster presentations, and a list of participants.

On the fourth day of the symposium, General Assembly Meeting of the International Association of Neuropterology was organized. A four-day post-symposium excursion (26-29 June) followed the formal scientific meeting. Participants visited the interesting collecting places located in the Regional Karst Park, Škocjan Caves system, River Soča Valley, and Triglav National Park in the Julian Alps.

The number of participants was higher than any recorded during the previous Symposia. Piran was an enchanting site and the convivial events (get together party at the Marine Biology Station, social dinner and excursions) received high praise from participants. Taking into account the number of participants at the Symposium,



**Slika 1: Participants of the 10<sup>th</sup> International Symposium on Neuropterology.**

the implemented professional and organisational work, and the first responses of the participants, it is possible to conclude that the scientific meeting succeeded as a professional event.

**Dušan Devetak**

#### ISKANJE ORODIJ ZA TRAJNOSTNO UPRAVLJANJE SEVERNEGA JADRANA



**Morje potrebuje naš glas**  
**The sea deserves our voice**

Zavod RS za varstvo narave je 7. in 8. oktobra 2008 v prostorih Morske biološke postaje Nacionalnega inštituta za biologijo pripravil mednarodno strokovno srečanje z naslovom *Vpliv človekovih dejavnosti na morju, morskem obrežju in zaledju na biotsko raznovrstnost Severnega Jadrana*.

Cilj posveta je bil prispevati k celostni obravnavi Severnega Jadrana, tako z vidika stanja morskih in obrežnih habitatnih tipov kot z vidika nabora človekovih dejavnosti in vplivov, njihovega posamičnega in predvsem kumulativnega učinka na morske in obrežne ekosisteme. Slednje je ob takšnih in drugačnih pritiskih urbanizacije, kmetijstva in industrije, kot so npr. plinski terminali, povečanje pomorskega prometa ipd., še posebej pomembno.

Naše ravnanje z morjem in morskim obrežjem v veliki meri odloča, kako in koliko bodo morje in njegove naravne vire lahko koristili naši zanamci. Odloča tudi o tem, ali bodo v tem skrajnem delu Sredozemlja še občudovali morske želve, delfine, podvodne travnike, leščurje, morske datlje. Nekoč smo se za neustrezno poseganje v morje in morsko obrežje izgovarjali na nepoznavanje, pomanjkanje podatkov in podobno. Danes to ne more in ne sme biti več izgovor.

Osrednja tema strokovnega srečanja je bil vpliv človekovih dejavnosti na biotsko raznovrstnost Severnega Jadrana. Predavanja oz. predstavitve in razprave so potekale v treh sklopih: *morski in obrežni habitatni tipi in vrste, pomorski promet in druge dejavnosti na morju in na kopnem ter zavarovana območja*.

Posveta so se udeležili številni znanstveniki in naravovarstveniki iz Slovenije, Hrvaške in Italije, s svojimi predavanji pa gostovali štirje tuji profesorji: Ferdinando Boero (Univerza Salento), Gerard Pergent (Univerza na Korziki), Alberto Basset (Univerza Salento) ter Nenad Smolaka (Inštitut Ruđer Bošković).

Severni Jadran je razmeroma plitev ekosistem, saj je njegova globina manjša od 50 m. Opredeljujejo ga stratifikacija vodnega stolpca, veliki rečni vnosi in visoka produktivnost, predvsem pa je to zelo občutljiv ekosistem, kajti navedenim značilnostim je treba dodati še izdaten ribolov, turizem, industrijo in pomorski promet. Leta, ko je lord Byron zapisal, da se človekov vpliv konča na morskem obrežju, so davno mimo in skrajni čas je, da pogledamo pod morsko gladino in se na lastne oči prepričamo o razdejanju, ki ga povzročamo. Spoznanje o nujnosti ohranjanja celovitosti morskega ekosistema, njegove raznolikosti in ekoloških procesov je pomembna osnova delovanja večine mednarodnih, vladnih in nevladnih organizacij, tudi tistih, ki se ukvarjajo z izkoriščanjem naravnih virov, od ribolova do izkoriščanja rudnin na morskem dnu in pod njim. Mednarodna skupnost pa si je zadala tudi dva pomembna cilja: da bo do leta 2010 ustavila upadanje biotske raznovrstnosti in da bo do leta 2012 na regionalnem in globalnem nivoju vzpostavljena reprezentativna mreža morskih zavarovanih območij.

Stališča, ki smo jih udeleženci posveta združili po bogati diskusiji, smo strnili v nekaj zaključkih:

Če želimo vedeti, kaj moramo zaščititi in ohraniti kot enkratno naravno dediščino tega najhladnejšega dela Sredozemlja, potrebujemo popis in oceno stanja biodiverzitete Severnega Jadrana.

Da bi lahko razvili ustrezne strategije za blažitev človekovih vplivov, potrebujemo meddržavne programe spremljanja ekološkega stanja morja, ki je nekakšen biološki filter za blaženje pritiskov, ki prihajajo iz urbanih, kmetijskih in industrijskih središč.

Nujno moramo povečati prizadevanja za varovanje tega dela, tako z vzpostavitvijo morskih zavarovanih območij kot z boljšim nadzorom zunaj teh območij.

Da bi o problemih Severnega Jadrana lahko bolje poročali odgovornim organom v jadranski ekoregiji, moramo zmanjšati fragmentacijo v jadranski znanstveni in akademski skupnosti.

Znanstveniki in naravovarstveniki želimo prednostno poudariti razvoj čezmejne (mednarodne) raziskovalne in znanstvene institucije za morsko in obalno biologijo in ekologijo, ki bi delovala kot virtualno središče in institucionalni okvir za mednarodno in medregijsko skupino raziskovalcev, profesorjev in študentov za študije in raziskave s področja biologije in ekologije Jadranskega morja.

Zbrane zaključke oz. stališča bomo kot priporočila pri odločitvah v prihodnosti posredovali Evropski uniji ter meddržavnim in nacionalnim organom s področja varovanja okolja, raziskav in ribištva.

Vsi prispevki s posveta skupaj z zaključki bodo zbrani v tematski 22. številki revije Varstvo narave, ki bo izšla v začetku leta 2009.

**Mateja Nose Marolt in Robert Turk**

**OBLETNICE  
ANNIVERSARI  
ANNIVERSARIES**

**BOTANIK TONE WRABER – SEDEMDESETLETNIK**



*Botanika na terenu ne ustavi niti vreme. Utrinek iz ekskurzije v Polhograjske Dolomite jeseni 2008, ki je bila del simpozija, prirejenega slavljenču v čast. (Foto: B. Frajman)*

Slovenski botanik Tone Wraber je letos dopolnil 70 let. Zdi se mi, da smo še nedavno praznovali njegovo šestdesetletnico, toda čas neusmiljeno teče naprej. Moj namen ni le, da se profesorja Toneta Wraberja spomnimo ob življenjskem jubileju, marveč tudi, da opozorim na bogato in vsestransko delovanje botanika, ki je bil in še vedno je moj botanični učitelj.

Profesor Wraber se je rodil 4. marca 1938 v Ljubljani v intelektualno bogatem okolju. Njegov oče je bil znani slovenski fitocenolog dr. Maks Wraber. Prav gotovo je to vplivalo na njegovo nadaljnjo pot, saj se je Tone "okužil" z ljubeznijo do rastlinskega sveta že zelo zgodaj. Represija takratne politike je utesnjevala svobodnejšega duha in drugačne nazore Tonetove družine. Kot otrok je doživel stiske; to pa je morda še podžgalo Tonetovo željo in silo k čim širši in čim globlji izobrazbi in razgledanosti, ki jo mlajši kolegi danes tako občudujemo pri njem. Njegov vodnik po naravi je bil poleg očeta tudi njegov skavtski učitelj, profesor Pavel Kuna-  
ver - Sivi Volk. Odtod tudi posebna ljubezen do gora, ki so Tonetu še vedno zelo blizu.

Po maturi 1956 na klasični gimnaziji v Ljubljani je v letih 1956–61 študiral biologijo na Ljubljanski univerzi. Po diplomi se je najprej zaposlil kot kustos v Prirodoslovnem muzeju Slovenije. Že leta 1963 je izšel njegov vodnik "Naše zaščitene rastline", prvo pomembnejše delo botaničnega naravovarstva do tedaj. Leta 1968 je bil imenovan za asistenta v Botaničnem vrtu

Ljubljanske univerze. To je bilo obdobje številnih terenskih ekskurzij po Sloveniji, katerih rezultat so bile nove najdbe za floro Slovenije in številna nova nahajališča redkih vrst. Profesor Wraber je skrbel za vestno in stalno zbiranje herbarijskega materiala za herbarijsko zbirko Ljubljanske univerze. O tem se prepričamo, če se nam ponudi priložnost brskati po herbarijskih polah: med posušenimi rastlinami, ki jih je zbral prof. Wraber, je sled prehojenih kotičkov po vsej Sloveniji in Balkanu pa vse do Afrike in Himalaje. Leta 1973 je Tone Wraber začel univerzitetno kariero kot asistent, danes pa je upokojeni redni profesor za taksonomijo rastlin in fitocenologijo. Upokojil se je pred tremi leti.

Profesorju Wraberju je bogato poznavanje jezikov in stroke omogočalo daljša študijska bivanja v tujini. Naj omenim, da se je že kot komaj triindvajsetleten mladenič izpopolnjeval pri ustanovitelju srednjeevropske fitocenološke šole, profesorju Braun-Blanquetu v Montpelieru v Franciji. Daljši čas je deloval na univerzi v Trstu pri vodilnem italijanskem botaniku Sandru Pignattiju, pod čigar mentorstvom je izdelal tudi doktorsko disertacijo o vegetaciji skalnih razpok v Julijskih Alpah leta 1972.

Z eksotičnimi ekskurzijami je začel že leta 1969, kot udeleženec jugoslovanske odprave na Anapurno, in 1972 na Makalu, večkrat je obiskal Nepal. Med čezmorskimi ekskurzijami je bila še posebno uspešna tista v Centralnoafriško republiko. Profesorju Wraberju ni tuja niti (pol)eksotika naše nekdanje skupne države – veliko energije in časa namreč posveča raziskovanju flore Balkana, predvsem Srbije, posebej Kosova, Makedonije in Črne gore, ter v zadnjih letih tudi Albanije. Profesor Wraber je nam, študentom, odkrival botanično najslabše poznane in najbolj zakotne predele takratne Jugoslavije; vselej pa je bil tudi vir podatkov o zgodovini, etnologiji in kulturi območja, skozi katerega smo potovali.

Če so ena izmed Wraberjevih ljubezni Alpe, je druga prav gotovo Mediteran. Po mami je Primorec in odličen poznavalec družbenih razmer v Istri, na Primorskem in v zamejstvu. V sedemdesetih in osemdesetih letih je botanično "ponovno odkril" Slovensko Istro: Osp, Strunjan in Ronek, Steno in Sv. Štefan v dolini Dragonje, Sečoveljske soline, Sv. Katarino pri Ankaranu, Kubed ... Da so ti enkratni ambienti danes zakonsko zavarovana območja, je tudi njegova zasluga. Slovenska zahodna meja ga ne zanima le botanično, marveč tudi z jezikovnega, narodnostnega in kulturnega stališča.

Tone Wraber je opisal nekaj za znanost novih taksonov (npr. *Gentiana lutea* subsp. *vardjani*, *Solenanthus krasniqi*), drugim je revidiral taksonomski status, našel je desetine novih vrst za floro Slovenije in nova nahajališča redkih in še bolj redkih vrst. Pri tem pa ne smemo pozabiti na Toneta Wraberja kot izkušenega fitocenologa klasične šole, ki se ukvarja predvsem z združbami nad gozdno mejo, s skalnimi razpokami in z melišči.

Tone Wraber je bil moj mentor (in seveda tudi številnim drugim slovenskim botanikom) pri srednješolski raziskovalni nalogi, diplomu, magisteriju in doktoratu. Njegova predavanja so bila vselej odlično pripravljena, podkrepljena z diapozitivi z vseh koncev sveta, obogaten z zanimivostmi, vendar ne na škodo podrobnosti in dejstev, ki so pri taksonomiji nujna. Na terenskih vajah je vsak študent odnesel toliko, kot ga je zanimalo, poleg botaničnih zanimivosti, pa je profesor Wraber vselej natrosil še kup zgodovinskih in krajevnih zanimivosti. Še leto dni ni od tega, ko je pri nas v Mariboru predaval študentom o Snežniku: ta gora ga zelo zanima še posebno zadnja leta, po odhodu v pokoj. O njeni flori in vegetaciji je objavil vrsto prispevkov v znanstveni in poljudni literaturi.

V svoji dosedanji karieri je bil urednik ali član uredniškega odbora Proteusa, Scopolije, Varstva narave, Hladnikije, koprskih Analov idr. Je tudi predsednik Botaničnega društva Slovenije, ki ima zametke v vsako-

letnem srečanju slovenskih botanikov; ta srečanja je spodbudil in organiziral prav profesor Wraber.

Je tudi aktiven član številnih mednarodnih združenj, njegova bogata bibliografija – če se omejimo le na strokovno in znanstveno – pa posega na področja floristike, taksonomije, botanične zgodovine, fitocenologije in varstva narave. Po padcu enopartijskega sistema je bil tudi nosilec pomembne visoke funkcije prorektorja Ljubljanske univerze.

Po upokojitvi se je prof. Wraber s soprogo preselil iz stanovanjskega bloka v središču Ljubljane v hišo v Polhovem Gradcu. Prav simbolično, saj je Polhov Gradec zaradi Blagajevga volčina botanično znamenit kraj! V čast in veselje mi je, da ob sedemdesetletnici zaželim profesorju Tonetu Wraberju enakega poleta in energije, kot ju je imel doslej, zdravja, ustvarjalnosti in veliko lepih trenutkov v naravi, tudi na domačem vrtu.

**Mitja Kaligarič**